

## Supplementary Information

# Targeting PI3K $\gamma$ Anchoring Enhances CFTR Membrane Localization and Modulator Efficacy via PKD1

Alessandra Murabito<sup>1†</sup>, Marco Mergiotti<sup>1†</sup>, Valeria Capurro<sup>2</sup>, Alessia Loffreda<sup>3</sup>, Mingchuan Li<sup>1</sup>, Paola Peretto<sup>1</sup>, Kai Ren<sup>1</sup>, Andrea Raimondi<sup>3</sup>, Carlo Tacchetti<sup>3,4</sup>, Dario Diviani<sup>5</sup>, Nicoletta Pedemonte<sup>2</sup>, Emilio Hirsch<sup>1,6</sup> and Alessandra Ghigo<sup>1,6\*</sup>

<sup>1</sup>Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino; Torino, Italy

<sup>2</sup>UOC Genetica Medica, IRCCS Istituto Giannina Gaslini; Genova, Italy

<sup>3</sup>Experimental Imaging Center, IRCCS Ospedale San Raffaele; Milan, Italy

<sup>4</sup>Università Vita-Salute San Raffaele, Milan, Italy

<sup>5</sup>Department of Biomedical Sciences, Faculty of Biology and Medicine, University of Lausanne; Lausanne, Switzerland

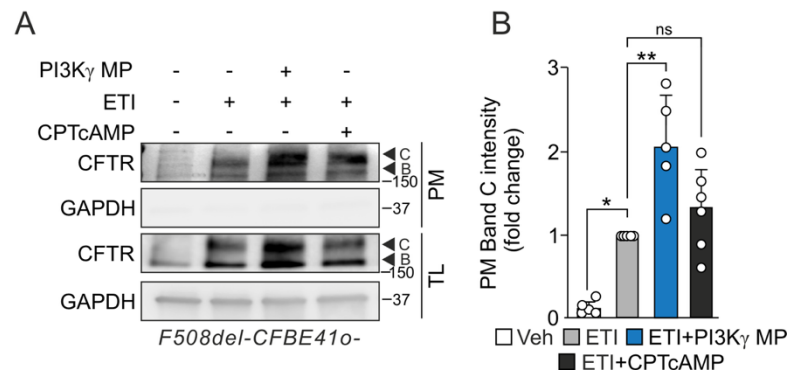
<sup>6</sup>Kither Biotech S.r.l.; Torino, Italy

†Equal contribution

\*Corresponding authors: Alessandra Ghigo, Via Nizza 52, 10126, Torino, ITALY.

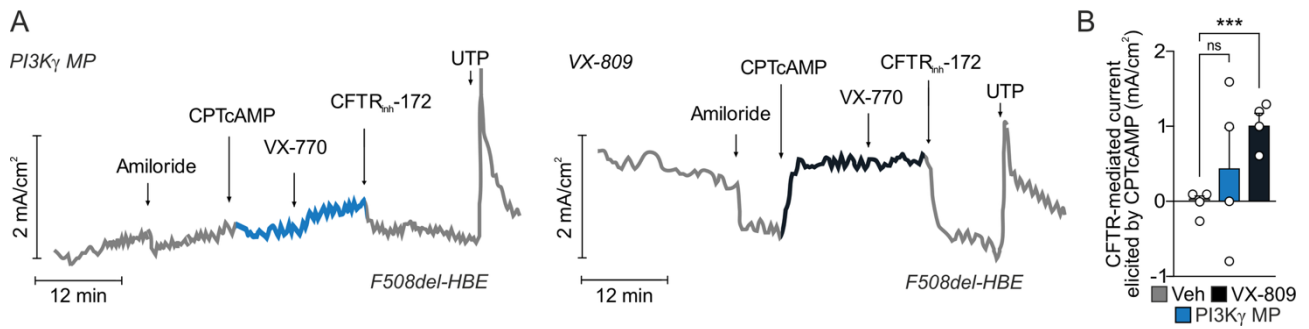
alessandra.ghigo@unito.it

## Supplementary Figures



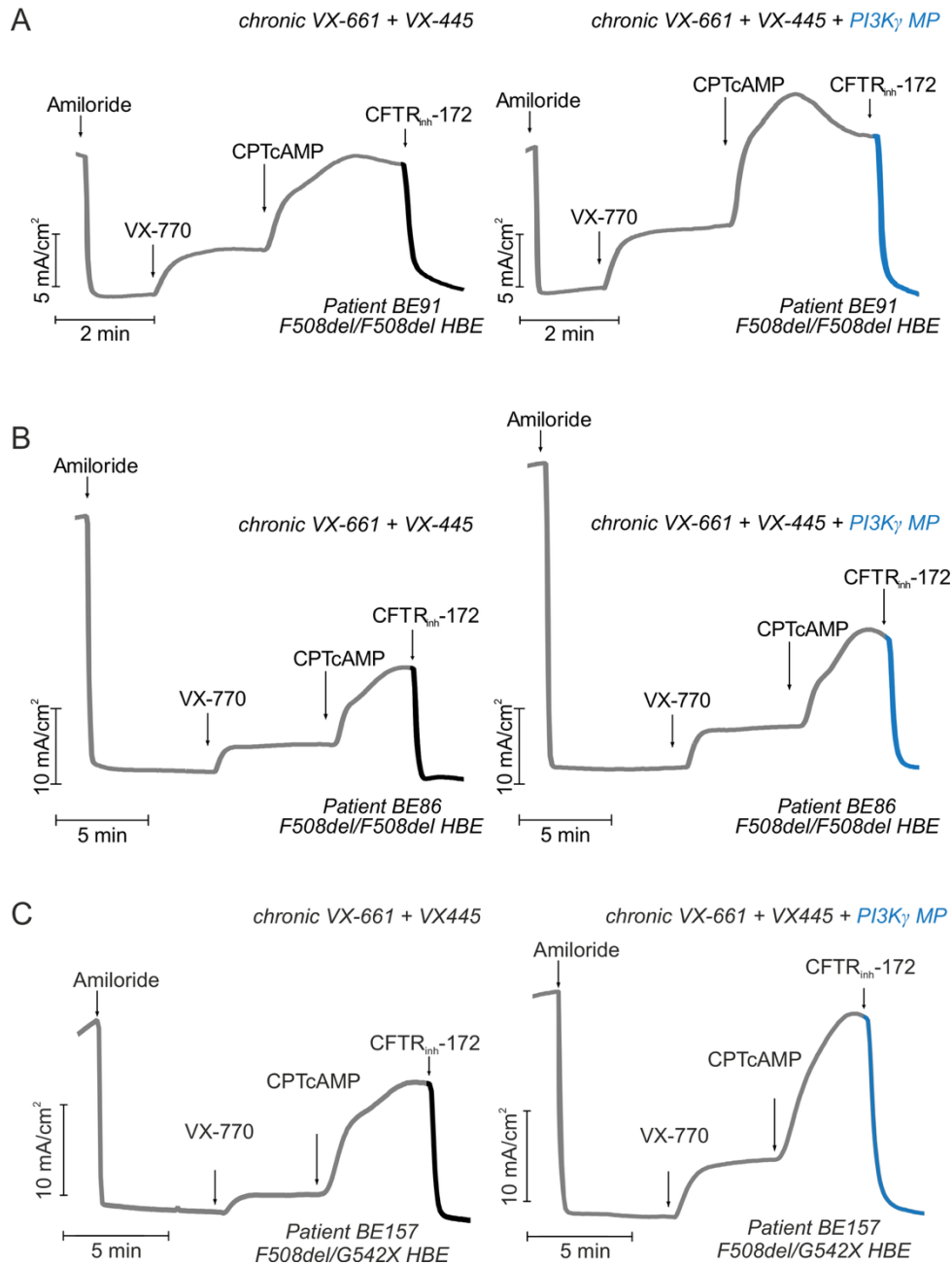
**Fig S1. Global cAMP elevation via chronic CPTcAMP treatment does not reproduce the effect of PI3K $\gamma$  MP on CFTR trafficking**

- A) Representative Western blot of CFTR and GAPDH in PM fractions and total lysates (TL) of *F508del-CFBE41o-* cells treated for 24 h with ETI (3  $\mu$ M VX-445, 10  $\mu$ M VX-661, and 1  $\mu$ M VX-770) alone or in combination with PI3K $\gamma$  MP (25  $\mu$ M) or CPTcAMP (100  $\mu$ M). GAPDH was absent from PM fractions and served as a loading control in TL.
- B) Quantification of PM CFTR band C intensity as in (A), presented as fold change relative to ETI alone. \*P < 0.05 and \*\*P < 0.01 by one-way ANOVA with Bonferroni's post-hoc test. Data are mean  $\pm$  SEM. Data points represent independent biological replicates.



**Fig S2. Chronic PI3K $\gamma$  MP treatment does not replicate the effect of the CFTR corrector VX-809**

- A) Representative short-circuit current ( $I_{SC}$ ) traces in primary human bronchial epithelial (HBE) cells from a F508del/F508del CF donor grown at the air-liquid interface (ALI). Cells were treated for 24 hours with either PI3K $\gamma$  MP (10  $\mu$ M), or the CFTR corrector VX-809 (3  $\mu$ M), followed by sequential exposure to amiloride (10  $\mu$ M), CPTcAMP (100  $\mu$ M), VX-770 (1  $\mu$ M), CFTR<sub>inh</sub>-172 (10  $\mu$ M), and UTP (100  $\mu$ M).
- B) Average CPTcAMP-stimulated current from n=4 technical replicates as in (A). \*\*\*P<0.001 by Student's t-test. Data are mean  $\pm$  SEM.



**Fig. S3. Effects of PI3K $\gamma$  MP in human bronchial epithelial cells from different F508del/F508del and F508del/G542X CF donors**

A-B) Representative short-circuit current ( $I_{sc}$ ) traces in primary human bronchial epithelial (HBE) cells from two F508del/F508del CF donors (patient BE91 and BE86) grown at the air-liquid interface (ALI). Cells were corrected for 24 h with 10  $\mu$ M VX-661 and 3  $\mu$ M VX-445, with or without PI3K $\gamma$  MP (10  $\mu$ M), followed by acute exposure to amiloride (100  $\mu$ M), VX-770 (1  $\mu$ M), CPTcAMP (100  $\mu$ M), and CFTR<sub>inh</sub>-172 (10  $\mu$ M).

C) Representative  $I_{SC}$  traces in primary HBE cells from a F508del/G542X CF donor (patient BE157) grown at the ALI. Cells were corrected for 24 h with 10  $\mu$ M VX-661 and 3  $\mu$ M VX-445, with or without PI3K $\gamma$  MP (10  $\mu$ M), followed by acute exposure to amiloride (100  $\mu$ M), VX-770 (1  $\mu$ M), CPTcAMP (100  $\mu$ M), and CFTR<sub>inh</sub>-172 (10  $\mu$ M).