Supplementary Information

Esc peptides as novel potentiators of defective cystic fibrosis transmembrane conductance regulator: an unprecedented property of antimicrobial peptides

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Supplementary Text

Null FRT cells
Null FRT cells were cultured in Coon’s modified Ham’s F-12 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Short-Circuit Current Recordings in primary bronchial epithelium.
Primary human bronchial epithelial were seeded on porous membranes (12 mm Snapwell inserts, Corning, code 3801) as described in the main text to form a differentiated epithelium under air-liquid condition. Epithelia were treated for 24 h with 0.1% DMSO as well as with 1 μM VX-809 or 10 μM Esc peptide. Epithelia were mounted in a vertical diffusion chamber, as described in the main text. The transepithelial voltage was short-circuited with the same voltage-clamp and the short-circuit current recorded as described in the main text. Afterwards, 10 μM amiloride was added; subsequently, CFTR channels were activated by phosphorylation upon addition of 100 μM CPT-cAMP. Then, 1 μM of the potentiator VX-770 was added on the epithelium apical side. Finally, 10 μM CFTR<sub>inh</sub>-172 was added to the epithelium apical side. Current measurements were done after 5 min from the addition of each substance.

Patch-clamp experiments in null FRT cells
Whole-cell membrane currents were recorded in null FRT cells not expressing CFTR by using the same experimental solutions and stimulation protocols as described in the main text for F508del- FRT.
**Fig. S1** Effect of peptides on F508del-CFTR activity in primary airway epithelial cells by the short-circuit current technique. **a-c** Representative traces from Ussing chamber recordings of bronchial epithelial cells (HBE) derived from a homozygous F508del patient after 24 h incubation with 0.1% DMSO and subsequent treatment with CPT-cAMP + VX-770 (a) or after incubation with 10 μM Esc(1-21) (b) or 1 μM VX-809 (c) and subsequent treatment with CPT-cAMP + VX-770. Pre-incubation of samples with Esc(1-21)-1c gave similar results to those of Esc(1-21) and therefore they are not shown. **d** Bar graphs indicate the CFTR-mediated current as the difference between the current measured in the epithelia treated with the indicated compounds before and after inhibition of CFTR by CFTR\textsubscript{Inh}-172 (10 μM). Data are the mean ± SEM from n=3 independent experiments. The level of statistical significance versus DMSO-treated samples is indicated as follows: *p<0.05. Comparison between data was done using Student’s t test. The solid black line indicates the delta current measured in epithelia treated with DMSO.
Fig. S2 Analysis of membrane patch in F508del-FRT cells from whole-cell patch technique. a Representative superimposed currents elicited at membrane potential from -80 to +120 mV in the control solution (top panel); after addition of 20 μM FSK+10 μM Esc(1-21) (central panel) and after subsequent addition of 10 μM CFTR[Inh-172] (bottom panel). b The same as a but for 10 μM Esc(1-21)-1c. c The same as a but for 50 μM GEN
Fig. S3 Membrane currents elicited in null FRT cells. a-c Representative whole-cell membrane currents from whole-cell patch-clamp experiments. The figure shows superimposed currents elicited at membrane...
potentials in the range from -80 to +120 mV in the control external solution (control, left panels) and after application of 20 μM FSK plus 10 μM of Esc peptides or GEN (right panels). d Time course of currents elicited in null FRT cells during addition of FSK+ 10 μM Esc peptide or GEN at different potentials. e Current ratio at +100 mV after application of FSK+ GEN or FSK + Esc peptides as indicated. Current intensity was normalized to that measured under control basal conditions (before addition of compounds). Stimulation with GEN or Esc peptides did not cause any increase of current in cells not expressing CFTR, and therefore it was not necessary to use the CFTR_{inh-172}. Data are expressed as mean ± SEM from n≥3 independent experiments.

**Fig. S4** Analysis of membrane patch in F508del-FRT cells from inside out patch technique. a Representative superimposed currents elicited at membrane potentials from -80 to +120 mV in the control solution (top panel); after addition of PKA+10 μM Esc(1-21) (central panel) and after subsequent addition of 10 μM CFTR_{inh-172} (bottom panel). b The same as a but for 10 μM Esc(1-21)-1c. c The same as a but for 10 μM [dLeu^{14}, dphosphoSer^{17}]Esc.
Fig. S5 Analysis of membrane currents in F508del-FRT cells from patch-clamp. 

a) Representative superimposed currents elicited by whole-cell configuration at membrane potentials from -80 to +120 mV in the control solution (left panel) and after addition of 20 μM FSK (right panel). 

b) Current ratio at +100 mV for indicated treatment (control basal condition, black bar; after CFTR activation by FSK, red bar). Current intensity was normalized to that measured under control basal conditions. Data are expressed as mean ± SEM from n≥3 independent experiments.

c) Representative superimposed currents elicited by inside-out configuration at membrane potential from -80 to +120 mV in the control solution (left panel) and after addition of 125 nM PKA (right panel). 

d) Current ratio at +100 mV for indicated treatment (control basal condition, black bar; after CFTR activation by PKA, red bar). Current intensity was normalized to that measured under control basal conditions. Data are expressed as mean ± SEM from n≥3 independent experiments.
Fig. S6 Electrostatic surface potential of F508del NBDs in complex with peptides. a NBDs/Esc(1-21); b NBDs[DLu^{14}DphosphoSer^{17}]Esc. The F508del NBDs are colored according with their electrostatic surface potential (red = negative charge; white = lipophilic/neutral; blue = positive charge); Esc(1-21) and [DLu^{14}DphosphoSer^{17}]Esc are shown as cartoons and lines and are colored orange and hot pink, respectively.