Expanded View Figures

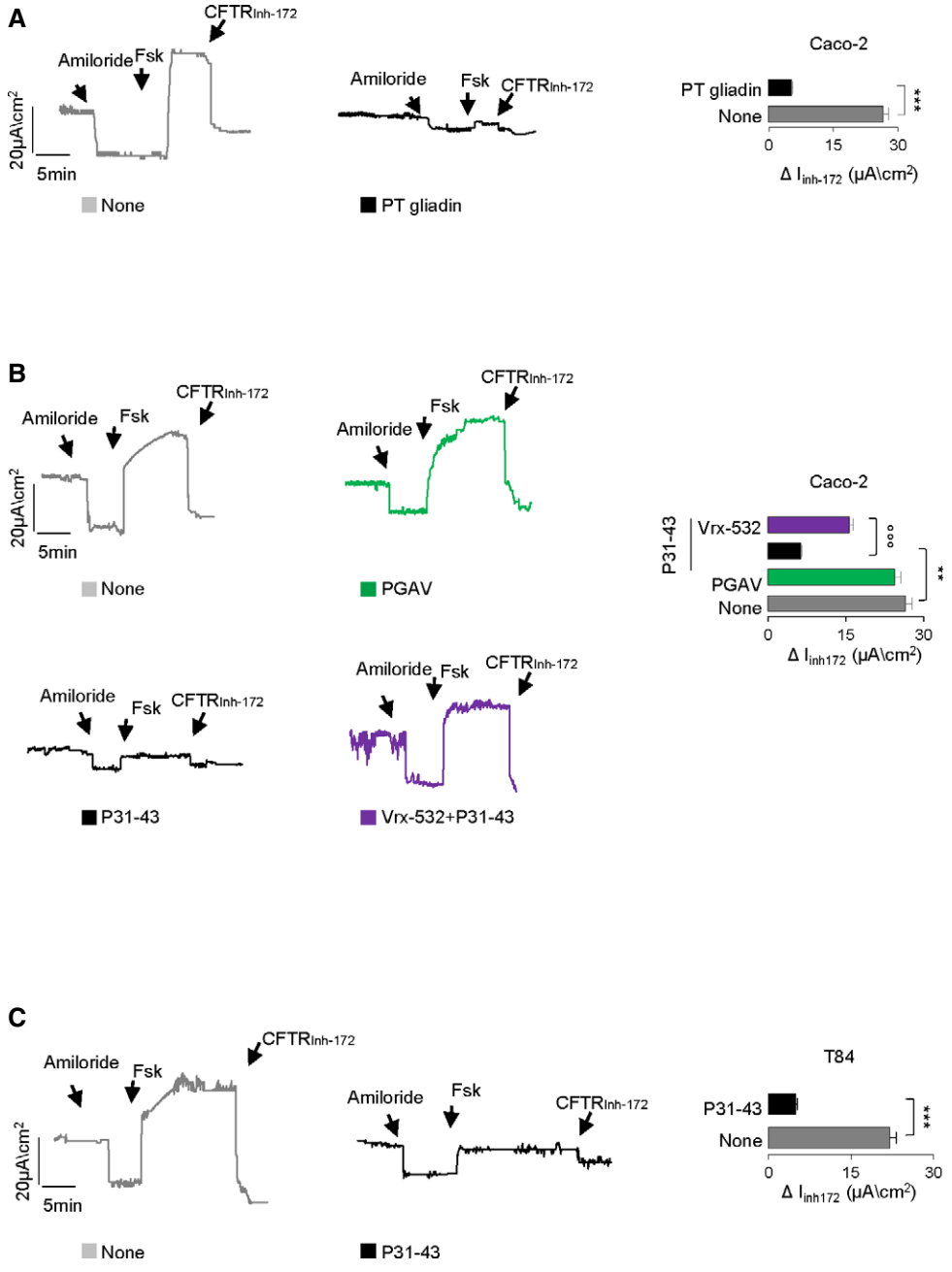


Figure EV1. Effects of gliadin and gliadin peptides on CFTR channel function in intestinal epithelial cells.

A–C Treatment of Caco-2 cells (A, B) or T84 (C) cells with a PT gliadin (500 μg/ml) (A) or P57–68 or PGAV or P31–43 (20 μg/ml; 3 h) in the presence or absence of pre-treatment with Vrx-532. CFTR-dependent Cl^- secretion measured by means of forskolin-induced (Fsk) increase in the chloride current [Isc (μA/cm^2)] in cells mounted in Ussing chambers; quantification of the peak CFTR Inhibitor 172 (CFTRinh172)-sensitive Isc (ΔIsc; n = 3 independent experiments). Mean ± SD of samples assayed. ***$P < 0.001$ vs. PT gliadin (A) or vs. P31–43 (C) or **$P < 0.01$ vs. P31–43 (B), °°°$P < 0.01$ vs. P31–43 + Vrx-532.

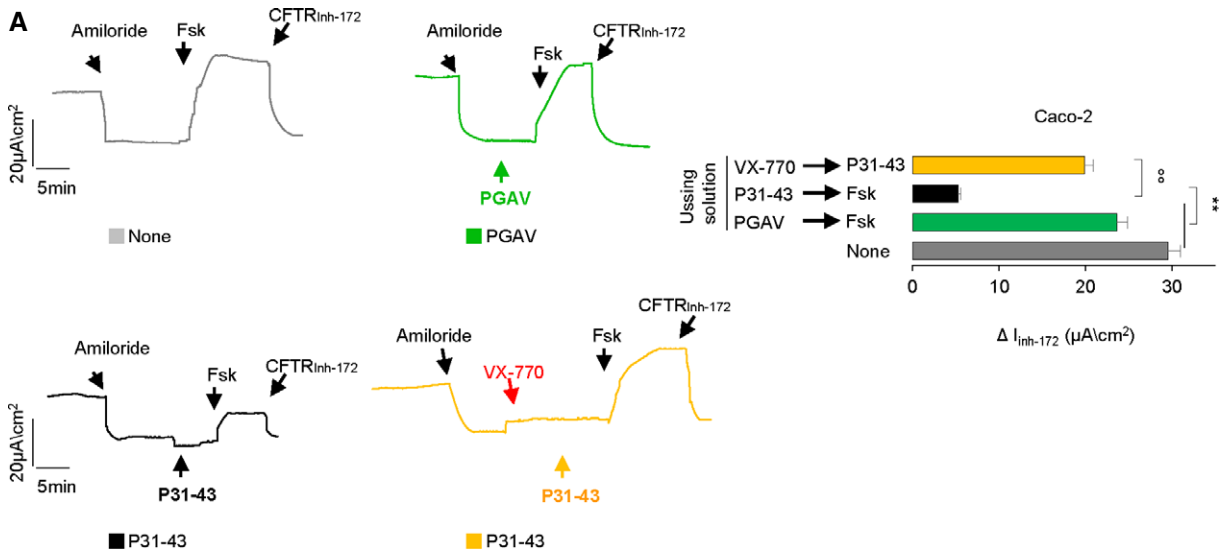
Source data are available online for this figure.

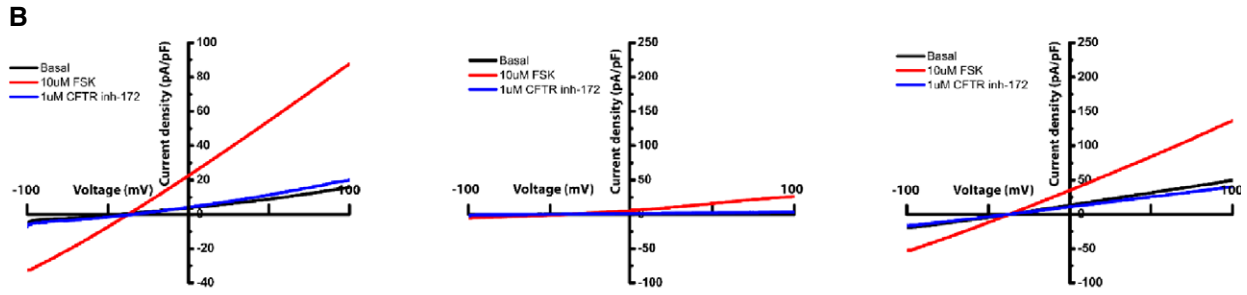
Figure EV2. Effects of P31–43 stimulation on forskolin-activated CFTR channel.

A Representative traces of CFTR-dependent Cl^- secretion measured by forskolin (Fsk)-inducible chloride current [Isc ($\mu A/cm^2$)] in Caco-2 cells mounted in Ussing chambers: effects of P31–43 (100 μM) and PGAV (100 μM) directly added to the bathing solution. After amiloride, the solution was directly supplied with buffer only (none, gray traces), with PGAV (green traces) or P31–43 (black traces) for 3–5 min (as detailed in Materials and Methods) and then pulsed with forskolin (Fsk; 20 μM) and finally with the CFTR inhibitor 172 ($CFTRinh_{172}$). Yellow traces: after amiloride, the solution was first supplied with VX-770 (10 μM) followed by P31–43 (100 μM) and then pulsed with forskolin (Fsk) followed by $CFTRinh_{172}$. Quantification of the peak CFTR inhibitor 172 ($CFTRinh_{172}$)-sensitive Isc (ΔIsc) in Caco-2 cells (n = 3 independent experiments, mean ± SD). $^{**}P < 0.01$ None or PGAV (gray or green traces) vs. P31–43 followed by Fsk (black traces), $^{\circ\circ}P < 0.01$ P31–43 followed by Fsk (black trace) vs. VX-770 followed by P31–43 and then by Fsk (yellow trace; ANOVA, Bonferroni *post hoc* test).

B Whole-cell CFTR current densities induced by 10 μM forskolin (Fsk) at +100 mV in Caco-2 cells w/o treatment (left), with 100 μM P31–43 peptide in the luminal solution during patch clamping (middle), or after treatment with 20 μM VX-770 (20 min), followed by an application of 100 μM P31–43 peptide in the luminal solution while patch clamping (right). Current–voltage (I–V) relationships were elicited by ramps from −100 mV to +100 mV (holding potential, −40 mV). Statistical analysis of average CFTR current densities induced by forskolin and blocked by CFTR inh-172 in cells treated as described. $n \geq 3$; $^{***}P < 0.001$ basal vs. P31–43, $^{\circ\circ\circ}P < 0.001$ P31–43 vs. VX770 + P31–43, two-way ANOVA with Bonferroni post-test. Data are presented as means ± SEM (bottom).

Source data are available online for this figure.


A
Amiloride
Fsk
CFTRInh-172
20μA\cm²
5min
None
PGAV
Caco-2
Ussing solution
VX-770
P31-43
Fsk
PGAV
None
°
**
0
10
20
30
Δ Iinh-172 (μA\cm²)
VX-770
P31-43


B
Basal
10uM FSK
1uM CFTR inh-172
Current density (pA/pF)
Voltage (mV)
-100
100
Basal
P31-43
VX-770 + P31-43

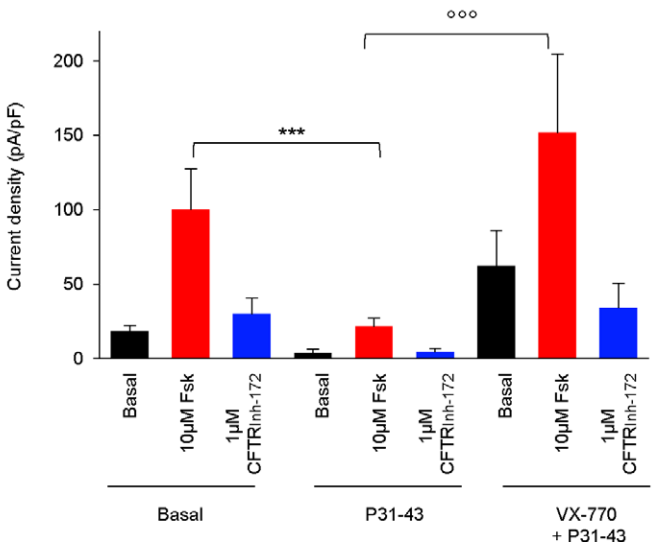

Current density (pA/pF)
200
150
100
50
0
***
°°°
Basal
10μM Fsk
1μM CFTRInh-172
Basal
P31-43
VX-770 + P31-43

Figure EV2.

Figure EV3. P31–43 interaction with NBD1.

A Caco-2 cells challenged with P31–43 peptide in the presence or absence of pretreatment of VX-770. Confocal analysis of endogenous CFTR interaction with P31–43 by proximity ligation assay (PLA). Scale bar, 10 μm. Quantifications were obtained from five randomly selected fields per condition, with each containing ~ 20–25 cells. Data are presented as means ± SEM, $^{\#}P < 0.05$ relative to VX-770 + P31–43; two-tailed unpaired Student's *t*-test.

B Caco-2 cells challenged with biotinylated P31–43 for 5 min at 37°C. Immunoprecipitation of purified protein from membrane fractions in non-reducing and non-denaturing conditions of CFTR protein and immunoblot with streptavidin-HRP or CFTR antibody (left). Immunoblot of purified protein from membrane fractions with anti-CFTR, anti-clathrin, and anti-EEA-1 antibodies. EEA-1 and clathrin were used as controls of purification of clathrin-positive membrane fraction vesicles (middle). Densitometric analysis of protein levels (right). Mean ± SD of three independent experiments.

C Caco-$2_{CFTR\text{-}KO}$ cells were transfected with pcDNA3.1_F400A/E403A-CFTR, pcDNA3.1_P439A/P477A-CFTR, or pcDNA3.b WT-CFTR plasmids (as positive control) or empty vector (as negative control). After 24 h, the cells were challenged with P31–43. Immunoprecipitation in non-reducing and non-denaturing conditions of CFTR protein and immunoblot with streptavidin-HRP or CFTR antibody ($n = 3$ independent experiments).

D Protein–protein docking and molecular dynamics of P31–43 (green) bound to CFTR.

E Comparison between the docking poses of P31–43 against CFTR structure (green) and against NBD1 alone (violet).

F 2D interaction map, between P31–43 peptide retrieved from the molecular docking procedure (PDB: 2BBO). Most important amino acids are detailed (blue circle for P31–43, red circle for NBD1).

Data information: The blots are representative of one experiment for group of treatment.
Source data are available online for this figure.

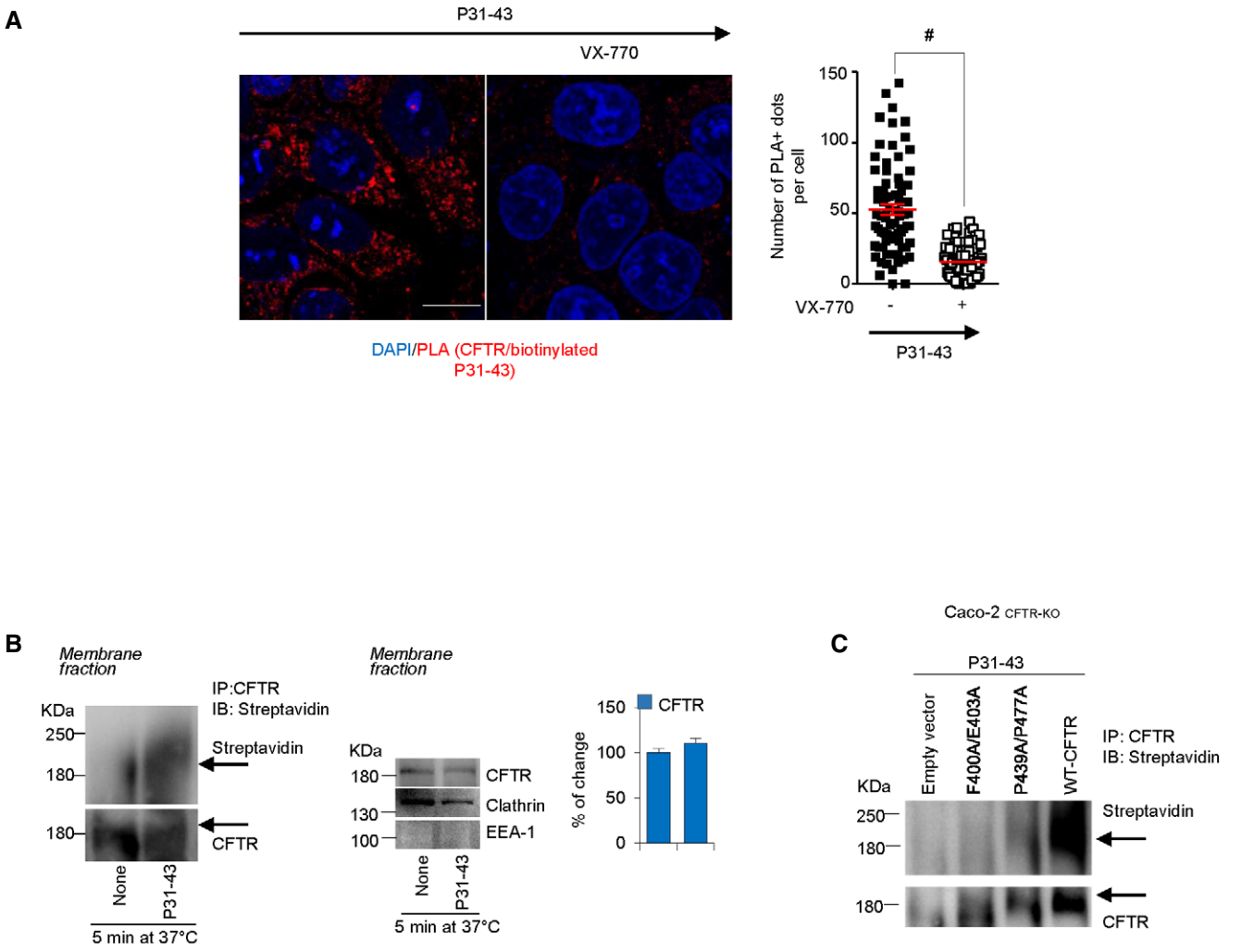

A
P31-43
VX-770
DAPI/PLA (CFTR/biotinylated P31-43)
Number of PLA+ dots per cell
#
150
100
50
0
VX-770
-
+
P31-43
B
Membrane fraction
IP:CFTR
IB: Streptavidin
KDa
250
180
Streptavidin
180
CFTR
None
P31-43
5 min at 37°C
Membrane fraction
KDa
180
CFTR
130
Clathrin
100
EEA-1
None
P31-43
5 min at 37°C
% of change
150
100
50
0
CFTR
C
Caco-2 CFTR-KO
P31-43
Empty vector
F400A/E403A
P439A/P477A
WT-CFTR
IP: CFTR
IB: Streptavidin
KDa
250
180
Streptavidin
180
CFTR
D
Transmembrane Domain
R Domain
P31-43
NBD2
NBD1
E
R Domain
P31-43
NBD1
F
P6
F400
P439
P477
P12
Q4
E403
Q10
W401
E402

Figure EV3.

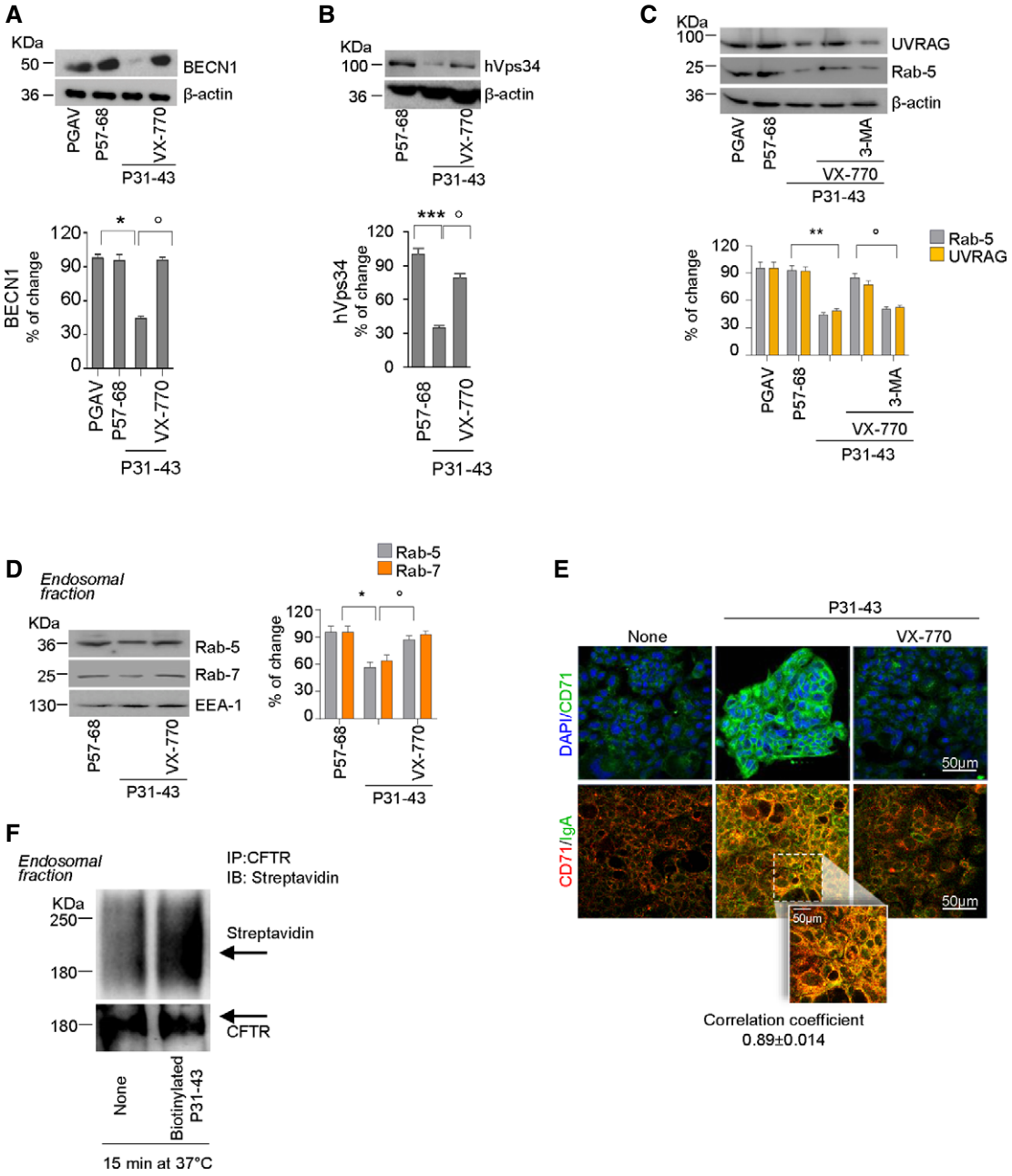


Figure EV4. Prevention by CFTR potentiators of P31–43 induced derangement of endosomal trafficking.

Caco-2 cells challenged with P57–68 or PGAV or P31–43 peptides in the presence or absence of pretreatment of VX-770 and 3-MA.

A–C Immunoblot of BECN-1 (A), phosphatidylinositol-3 (PI3)-kinase (hVps34) (B), UVRAG and Rab5 (C), and β-actin; densitometric analysis of protein levels (bottom). Mean ± SD of triplicates of independent experiments. *$P < 0.05$ or **$P < 0.01$ or ***$P < 0.01$ vs. P31–43, °$P < 0.05$ VX-770+P31–43 vs. VX-770 + 3-MA+P31–43 (ANOVA, Bonferroni *post hoc* test).

D Immunoblot of Rab5 and Rab7 proteins in endosomal protein fractions from Caco-2 cells challenged as described; endosomal antigen-1 (EEA-1) as loading control. Densitometric analysis of immunoblot is mean ± SD of triplicates of independent experiments. *$P < 0.05$ vs. P31–43, °$P < 0.05$ vs. P31–43 + VX-770.

E Caco-2 cells pre-incubated for 1 h at 4°C with S-IgA from hColostrum and then challenged with P31–43 in the presence or absence of VX-770. Confocal images of apical localization of CD71. Staining with anti-CD71 antibody and DAPI nuclear counterstaining (top) or anti-CD71 (red) and S-IgA (green) antibodies (bottom). Scale bar, 50 μm.

F Immunoprecipitation of CFTR protein in endosomal protein fractions in non-reducing and non-denaturing conditions, after 15 min of challenge with P31–43 and immunoblot with HRP-streptavidin or CFTR.

Data information: The blots are representative of one experiment for group of treatment.
Source data are available online for this figure.

Figure EV5. Prevention by VX-770 of gliadin-induced effects in gliadin-sensitive mice.

A–C BALB/c mice were fed for at least three generations with a gluten-free diet, orally challenged with vehicle or gliadin for 4 weeks (5 mg/daily for 1 week and then 5 mg/daily thrice a week for 3 weeks) in the presence or absence of intraperitoneal VX-770 administered 15 min prior gliadin challenge (n = 10 mice per group of treatment). (A and C) Immunoblot with antibodies against NHERF-1 and ezrin (A) or TG2 (C) and β-actin as loading control, in whole lysates from small intestine homogenates. Right panels, densitometric analysis of protein relative to β-actin, mean ± SD of triplicates of independent pooled samples. $^{**}P < 0.01$ vs. gliadin, $^{\circ}P < 0.05$ vs. VX-770, (ANOVA, Bonferroni *post hoc* test). (B) Confocal images of small intestine stained with antibodies against occludin (red) with nuclear counterstaining (blue). Scale bar, 100 μm.

D–G NOD mice (D and E) or NOD.scid AB0nullDQ8 mice (NOD-DQ8 mice) (F and G) challenged with gliadin for consecutive 4 weeks in the presence or absence of intraperitoneal administration of VX-770 15 min prior gliadin challenge (n = 10 mice per group of treatment). (D and F) Assessment in Ussing chambers of CFTR-dependent Cl^- secretion measured by forskolin-induced (Fsk) increase in the chloride current [Isc ($\mu A/cm^2$)]; quantification of the peak CFTR inhibitor 172 (CFTRinh172)-sensitive Isc (ΔIsc; n = 5). Mean ± SD of samples assayed. $^{***}P < 0.001$ vs. VX-770 treatment (Student's t-test). (E) IL-15, IL-17A, and IFN-γ protein levels from small intestine homogenates of NOD mice. Mean ± SD of triplicates of independent pooled samples. $^{***}P < 0.001$ vs. gliadin, $^{\circ\circ}P < 0.01$ vs. VX-770 treatment (ANOVA, Bonferroni *post hoc* test). (G) IFN-γ protein levels from small intestine homogenates of NOD-DQ8 mice. Mean ± SD of triplicates of independent pooled samples. $^{**}P < 0.01$ vs. gliadin, $^{\circ}P < 0.05$ vs. VX-770 treatment (ANOVA, Bonferroni *post hoc* test).

Data information: The blots are representative of one experiment for group of treatment.
Source data are available online for this figure.

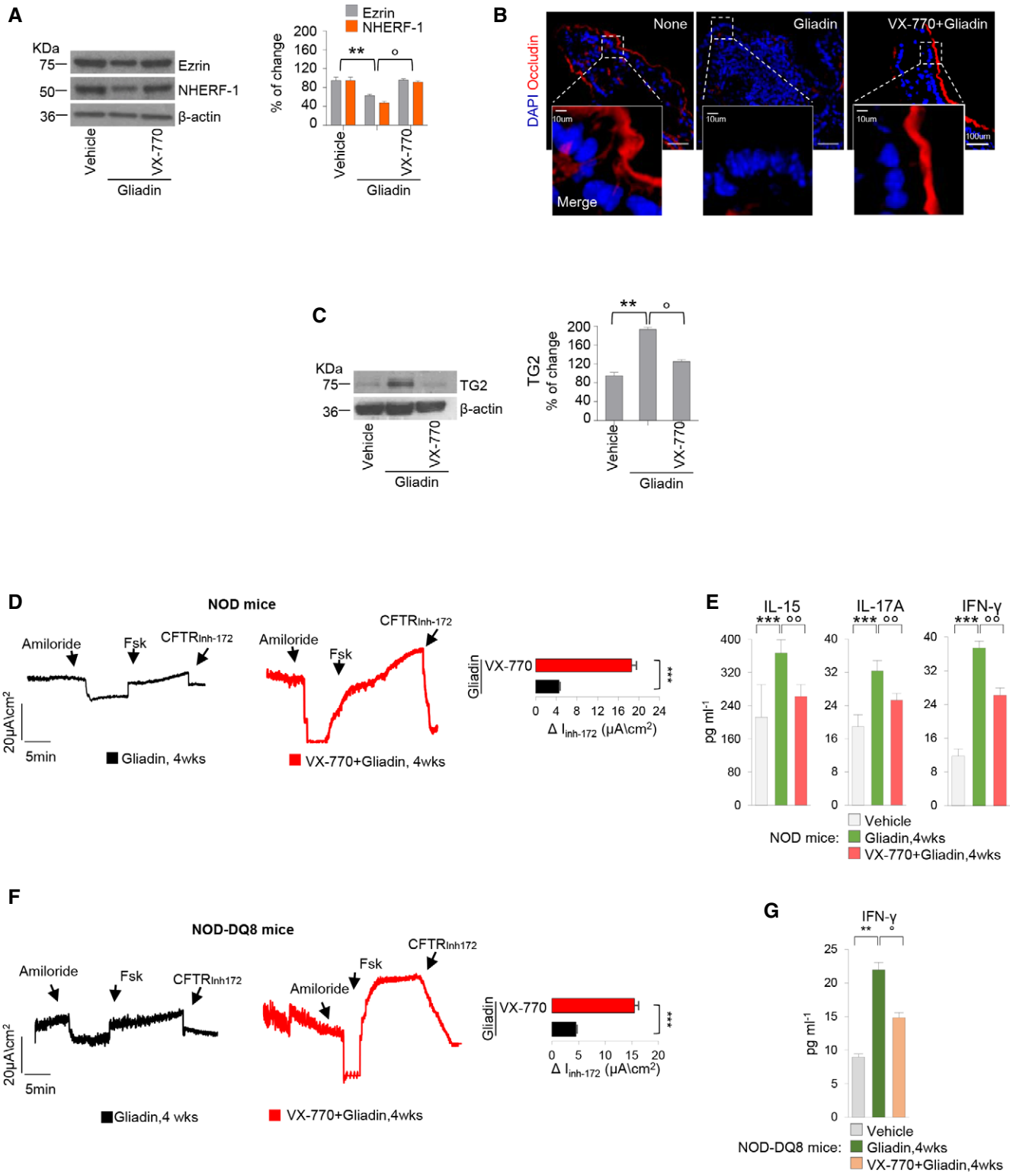


Figure EV5.