Figure S1

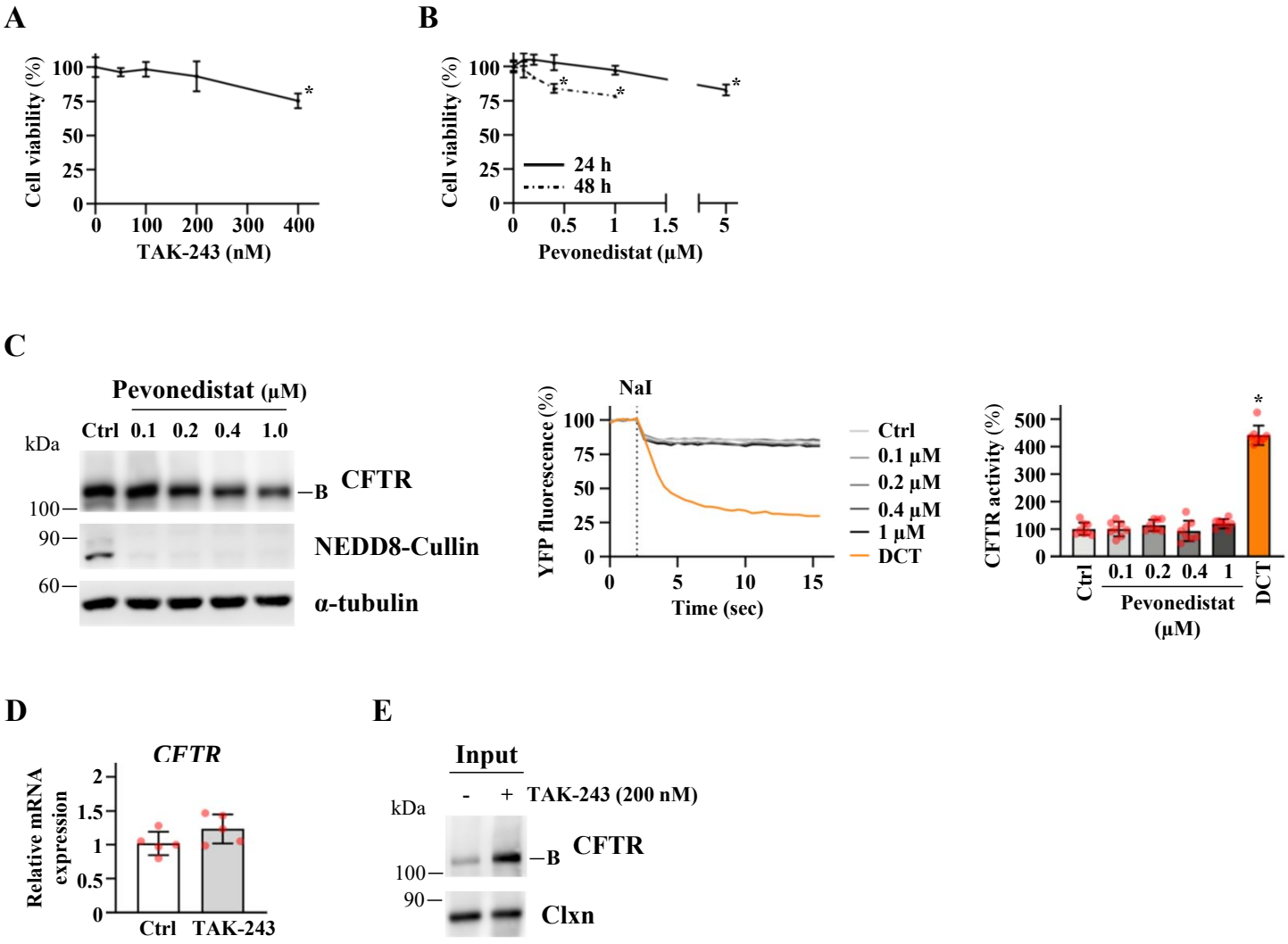
A
Cell viability (%)
100
75
50
25
0
0
100
200
300
400
TAK-243 (nM)
*
B
Cell viability (%)
24 h
48 h
0
0.5
1
1.5
5
Pevonedistat (μM)
C
Pevonedistat (μM)
Ctrl
0.1
0.2
0.4
1.0
kDa
100
90
60
B
CFTR
NEDD8-Cullin
α-tubulin
NaI
YFP fluorescence (%)
Time (sec)
Ctrl
0.1 μM
0.2 μM
0.4 μM
1 μM
DCT
CFTR activity (%)
Pevonedistat (μM)
D
CFTR
Relative mRNA expression
Ctrl
TAK-243
E
Input
TAK-243 (200 nM)
CFTR
Clxn

Fig. S1. (A) The viability of F508del-CFTR expressing CFBE41o- cells was assessed by the MTT assay after 24 h treatment with increasing concentrations (25, 50, 100, 200, or 400 nM) of TAK-243 and expressed as a percentage of control (means ± SD values, $n = 5$ (A), $n = 3$ (B); $*p < 0.05$ *vs* control). **(B)** The viability of F508del-CFTR expressing CFBE41o- cells was assessed by the MTT assay after 24 h treatment with increasing concentrations of Pevonedistat after 24 and 48 h treatment with increasing concentrations (0.1, 0.2, 0.4, 1, 5 μM for 24 h; 0.1, 0.2, 0.4, 1 μM for 48 h) and expressed as a percentage of control. (means ± SD values, $n = 5$ (A), $n = 3$ (B); $*p < 0.05$ *vs* control). **(C)** Immunoblot analysis of CFTR and NEDD8-Cullin in whole lysates from F508del-CFTR expressing CFBE41o- cells treated with DMSO (Ctrl) or increasing concentrations of Pevonedistat for 48 h. α-tubulin was used as loading control ($n = 3$) (left panel). Assessment of F508del-CFTR activity was carried out by HS-YFP assay in F508del-CFTR expressing CFBE41o- cells treated as indicated above or with double corrector treatment (DCT = 10 μM VX-661 + 3 μM VX-445) for 24 h. Centre panel exhibits representative traces measuring YFP quenching ($n = 4$), right panel shows the CFTR activity as a percentage of control (Scr) (means ± SD values, $n = 8$; $*p < 0.05$ *vs* Ctrl). **(D)** *CFTR* mRNA level determined by quantitative real-time PCR in F508del-CFTR expressing CFBE41o- cells were treated with DMSO (Ctrl) or TAK-243 (200 nM) for 24 h. CFTR mRNA expression was normalized to 18S RNA and reported relative to its expression in Ctrl cells that was arbitrarily set to 1 (means ± SD values, $n = 5$). **(E)** F508del-CFTR expressing CFBE41o- cells were treated with DMSO or 200 nM TAK-243 for 24 h, lysed and analysed by western blot with anti-CFTR antibody. Calnexin (Clxn) was used as a loading control. The lysates were used for the immunoprecipitation experiments of Fig. 2D.

Figure S2

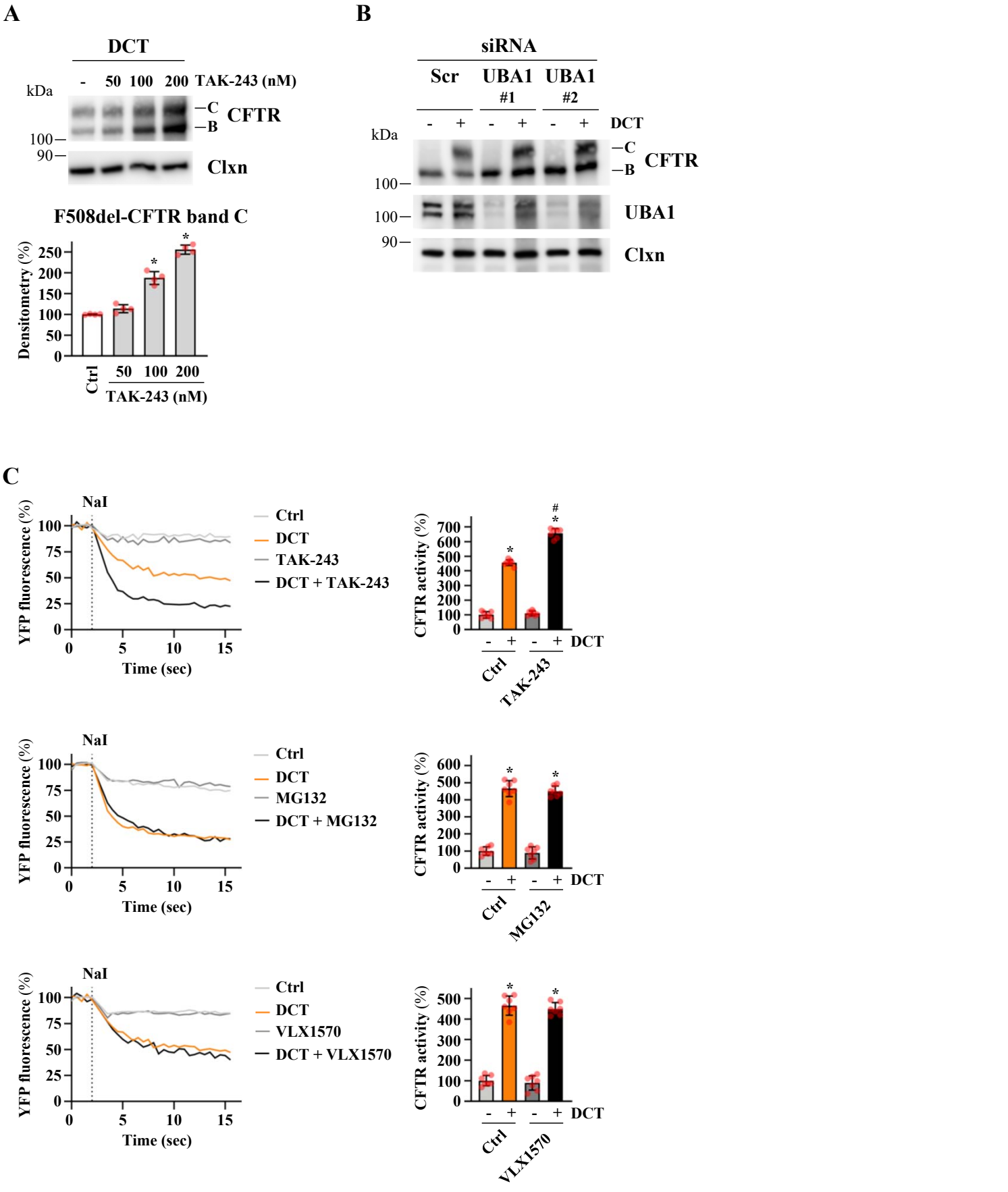
A
DCT
- 50 100 200 TAK-243 (nM)
kDa
100
90
C
B
CFTR
Clxn
F508del-CFTR band C
Densitometry (%)
250
200
150
100
50
0
Ctrl
50 100 200
TAK-243 (nM)
B
siRNA
Scr
UBA1 #1
UBA1 #2
- + - + - + DCT
kDa
100
100
90
CFTR
UBA1
Clxn
C
NaI
YFP fluorescence (%)
Time (sec)
Ctrl
DCT
TAK-243
DCT + TAK-243
CFTR activity (%)
DCT
Ctrl
TAK-243
MG132
DCT + MG132
VLX1570
DCT + VLX1570

Fig. S2. (A) Immunoblot analysis of CFTR in whole lysates from F508del-CFTR expressing CFBE41o- cells treated with increasing concentrations of TAK-243 (50, 100, 200 nM) in combination with double corrector treatment (DCT = 10 μM VX-661 + 3 μM VX-445) for 24 h. Calnexin (Clxn) was used as a loading control. The figure panel is representative of four independent experiments. Lower panel shows the densitometric quantification of the immunostained F508del-CFTR band C. The values for CFTR band C are expressed as a percentage of the control cells (means ± SD values, $n = 4$; *$p < 0.05$ *vs* Ctrl). **(B)** F508del-CFTR expressing CFBE41o- cells were transfected with non-specific siRNA (Scr), or two different UBA1 specific siRNAs. After 24 h post-transfection cells were treated with DCT (10 μM VX-661 + 3 μM VX-445) for further 24 and lysed. Lysate proteins were analysed by western blot with the indicated antibodies. Calnexin (Clxn) was used as a loading control. The figure panel is representative of three independent experiments. **(C)** Assessment of F508del-CFTR activity was carried out by HS-YFP assay in F508del-CFTR expressing CFBE41o- cells treated with DMSO (Ctrl) or TAK-243 (200 nM) or MG132 (1 μM) or VLX1570 (250 nM) in combination (+) or not (-) with double corrector treatment (DCT = 10 μM VX-661 + 3 μM VX-445) for 24 h. Left panels exhibit representative traces measuring YFP quenching, right panels show the CFTR activity as a percentage of control cells not treated with DCT (Ctrl) (means ± SD values, $n = 7$; *$p < 0.05$ *vs* Ctrl, #$p < 0.05$ *vs* Ctrl with DCT).

Figure S3

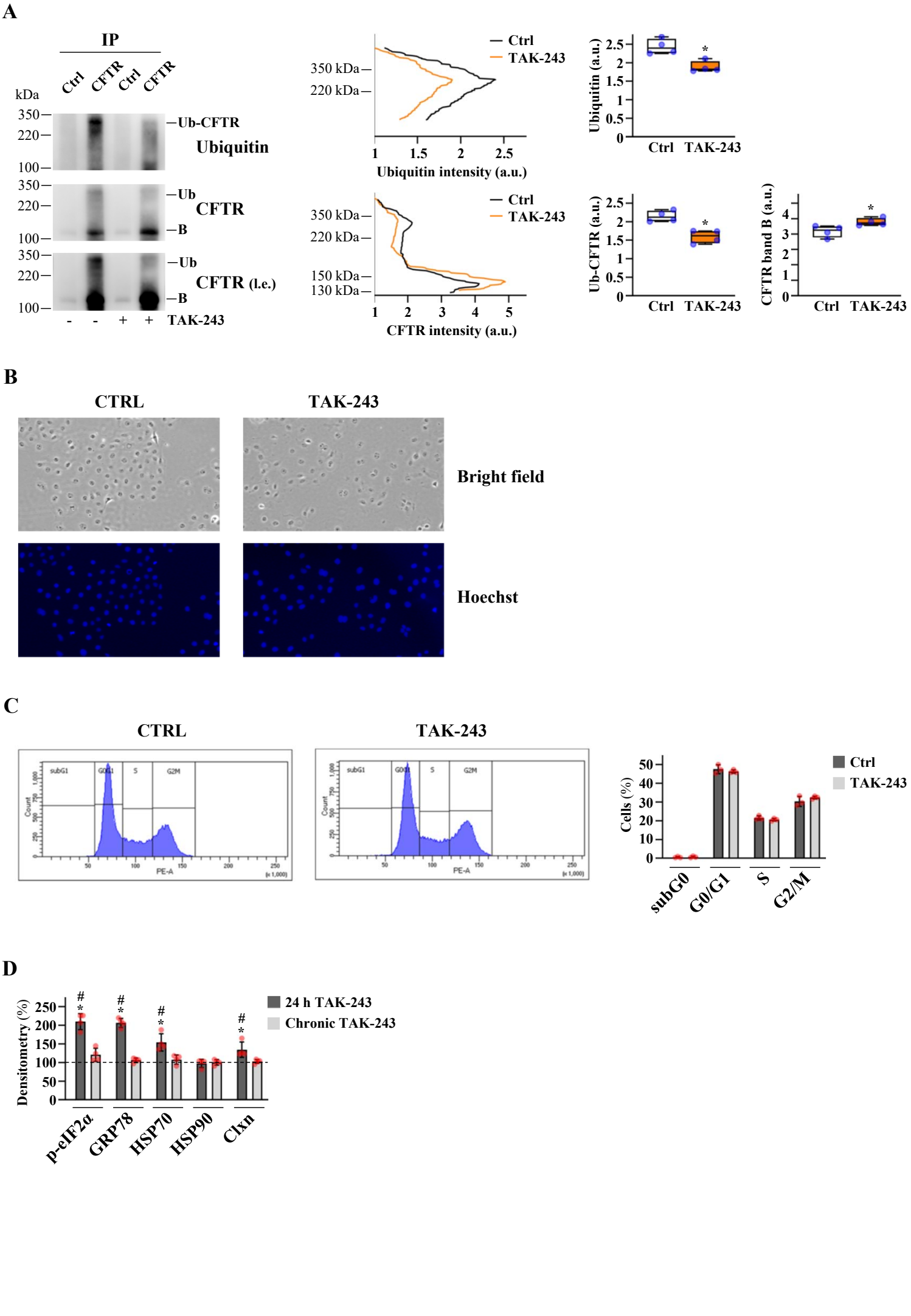
A
IP
Ctrl
CFTR
Ctrl
CFTR
kDa
350
220
100
Ub-CFTR
Ubiquitin
Ub
CFTR
B
Ub
CFTR (l.e.)
B
- - + + TAK-243
Ctrl
TAK-243
350 kDa
220 kDa
Ubiquitin intensity (a.u.)
150 kDa
130 kDa
CFTR intensity (a.u.)
Ubiquitin (a.u.)
Ub-CFTR (a.u.)
CFTR band B (a.u.)
B
CTRL
TAK-243
Bright field
Hoechst
C
CTRL
TAK-243
subG1
G0/G1
S
G2M
Count
PE-A
Cells (%)
subG0
G0/G1
S
G2/M
D
24 h TAK-243
Chronic TAK-243
Densitometry (%)
p-eIF2α
GRP78
HSP70
HSP90
Clxn

Fig. S3. (A) 300 µg of lysate proteins from F508del-CFTR expressing CFBE41o- cells untreated (Ctrl) or grew for at least 1 month in presence of TAK-243 (10 nM) were immunoprecipitated with a control antibody from the same class (Ctrl) or anti-CFTR (CFTR) antibody. The immunocomplexes were analysed by western blot with the indicated antibodies (left panel). The figure panel is representative of four independent experiments. A long exposition of CFTR detection is also shown (l.e.: long exposition). The central panels show representative density profiles of CFTR and ubiquitin in the CFTR-immunoprecipitated samples. Quantification of the density profiles was performed in the right panels by integrating the profile curves in the indicated intervals of molecular weight (Ubiquitin: 220-350 kDa; Ub-CFTR: 220-350 kDa; CFTR band B: 130-150 kDa) (means ± SD; n = 4; **p* < 0.05 *vs* Ctrl). **(B)** Nuclear staining (Hoechst) of F508del-CFTR expressing CFBE41o- cells chronically treated (at least 1 months) with DMSO (Ctrl) or TAK-243 (10 nM) magnification 20×. **(C)** Cell cycle analysis analysis by flow cytometry of F508del-CFTR expressing CFBE41o- cells chronically treated (at least 1 months) with DMSO (Ctrl) or TAK-243 (10 nM). On the right histogram data of the cell cycle analysis (means ± SD values, *n* = 3). **(D)** Densitometric quantification of the immunoblots of Fig. 4E. The values are expressed as a percentage of the control cells (dashed line) (means ± SD values, *n* = 4; **p* < 0.05 *vs* Ctrl; #*p* < 0.05 *vs* chronic treated cells).