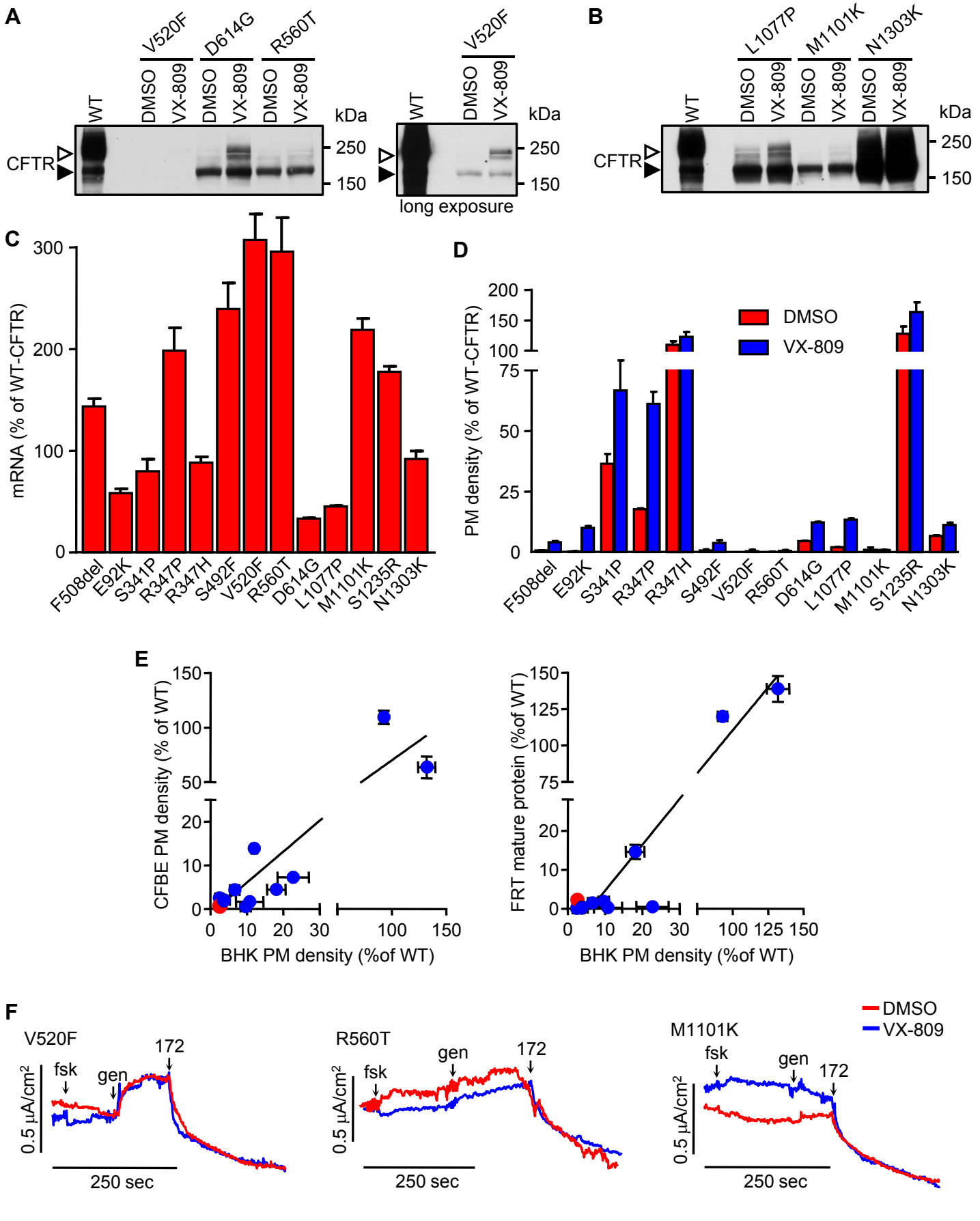
A
WT
V520F
D614G
R560T
DMSO
VX-809
CFTR
kDa
250
150
long exposure
B
L1077P
M1101K
N1303K
C
mRNA (% of WT-CFTR)
F508del
E92K
S341P
R347P
R347H
S492F
V520F
R560T
D614G
L1077P
M1101K
S1235R
N1303K
D
PM density (% of WT-CFTR)
E
CFBE PM density (% of WT)
BHK PM density (%of WT)
FRT mature protein (%of WT)
F
V520F
R560T
M1101K
fsk
gen
172
0.5 µA/cm²
250 sec

Figure E3. The biogenesis of CFTR2 mutants is similar in different cell models. **(A, B)** Immunoblot of CFBE expressing inducible CFTR variants with an extracellular 3HA tag under the control of the TetON doxycycline (dox) regulated transactivator. Expression was induced with 500 ng/ml dox for 3 days. The cells were incubated for 24 hours with DMSO or VX-809 (3 μM). CFTR was visualized with anti-HA antibody, and anti–Na^+/K^+-ATPase antibody served as loading control. The empty arrowheads show the mature, complex glycosylated CFTR (C-band), and the filled arrowhead show the immature, core glycosylated protein (B-band). These immunoblots are identical to those in Fig. 2B, but with longer exposure to allow the detection of low expression of band B and C for D614G, R560T and V520F-CFTR (A) as well as L1077P and M1101K-CFTR (B) **(C)** CFTR2 mutant mRNA levels in CFBE were determined by RT-qPCR as described in supplemental methods. **(D)** PM density measurements of CFTR2 mutant expression were performed by cell-surface ELISA, with and without 24h correction with VX-809 (3μM). The PM density normalized by cell viability is expressed as % of WT DMSO treated cells. **(E)** The correlation between PM density of CFTR mutants expressed in CFBE cells, was plotted against the PM density of CFTR mutants (F508del (red), CFTR2 mutants (blue)) expressed in BHK cells (*left*, $R^2 = 0.7582$, $p = 0.0002$). The correlation between mature protein (C-band) of CFTR mutants expressed in FRT cells (% of WT mature protein), as published by Van Goor et al. (1) was plotted against the PM density of CFTR mutants expressed in BHK cells (*right*, $R^2 = 0.9671$, $p < 0.0001$). **(F)** Representative I_{sc} traces of CFBE cells expressing the indicated CFTR2 mutants. Cells grown on filter supports were incubated for 24h with DMSO (red traces) or VX-809 (3 μM, blue traces). I_{sc} of mutant CFTR was stimulated with forskolin (fsk, 20 μM) and genistein (gen, 100 μM), followed by inhibition of CFTR with $CFTR_{inh}$-172 (20 μM). Measurements were performed in the presence of a basolateral-to-apical chloride gradient after basolateral permeabilization with amphotericin B (100 μM). All experiments are n=3; error bars are SEM.