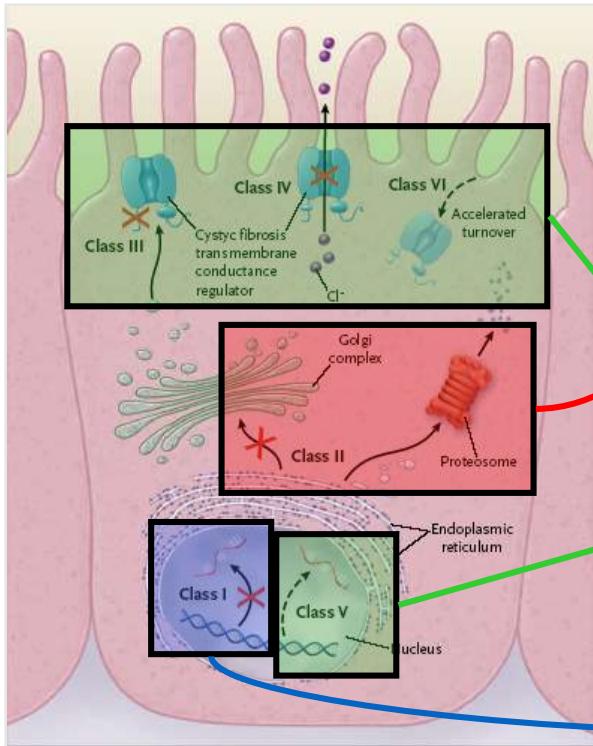


# Topics related to understanding and overcoming CFTR misfolding

Eric J. Sorscher, MD  
Emory University  
Atlanta, Georgia

November 6, 2015

# Therapeutic Approaches by Class

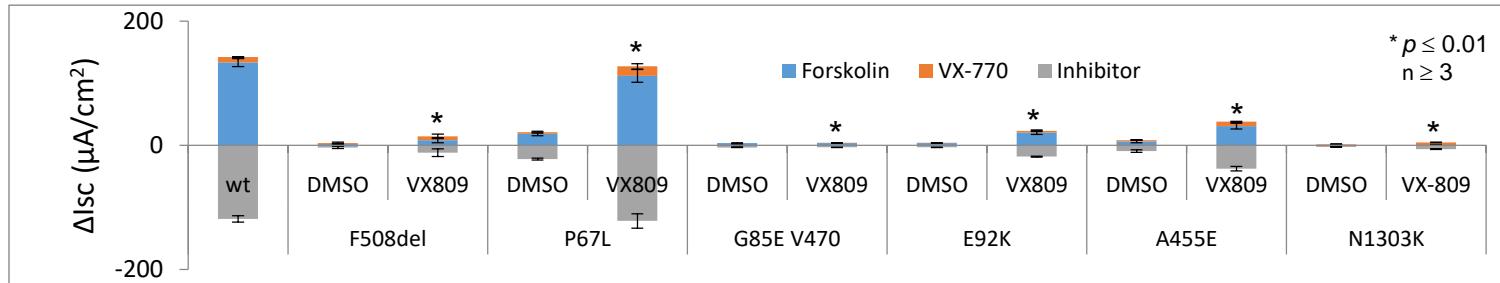
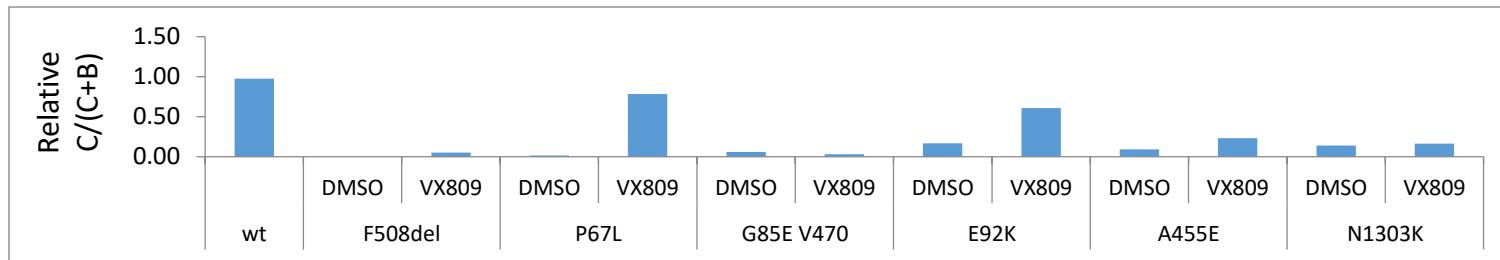
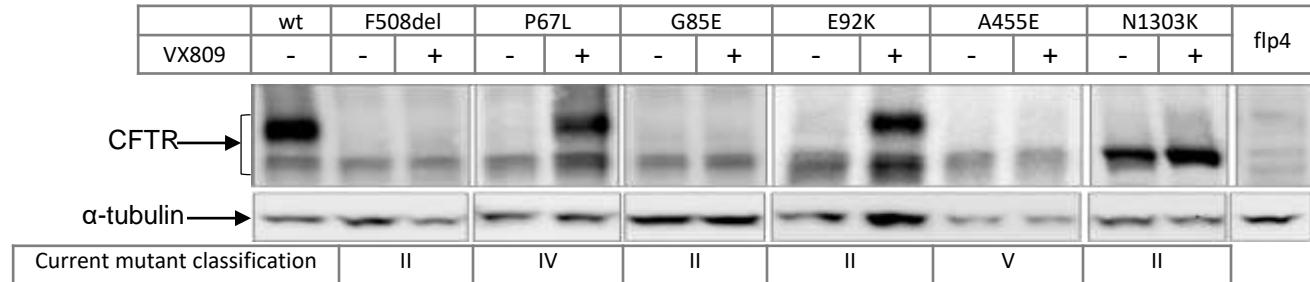


F508del CFTR  
Processing Corrector

CFTR Potentiators  
e.g. G551D

Translational  
Readthrough  
e.g. W1282X

# Diversity of corrector effect



FRT cells  
3  $\mu$ M VX809  
J. Hong

# P67L- A molecular case study

CLINICAL FEATURE (RANGE IN INDIVIDUALS WITHOUT CF)	AVERAGE OF ALL PATIENTS WITH MUTATION P67L	AVERAGE OF ALL PATIENTS
Sweat Chloride  (non-CF is less than 40mEq/L in children and older, less than 30mEq/L in infants)	61	96
Lung Function expressed as % predicted (non-CF 80%-120% predicted)	 	
Pancreatic Insufficiency  (less than 1% of non-CF expected to be PI)	33 %	85 %
Pseudomonas  (less than 1% of non-CF expected to have Pseudomonas)	47 %	55%
Average Age	28	20

## CFTR2 DATABASE

239/177,328 alleles

238/88,664 patients

## FOLDING/FUNCTION

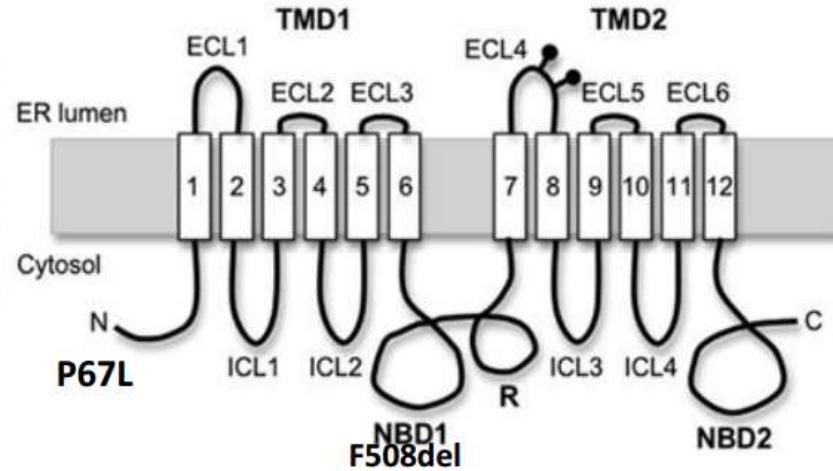
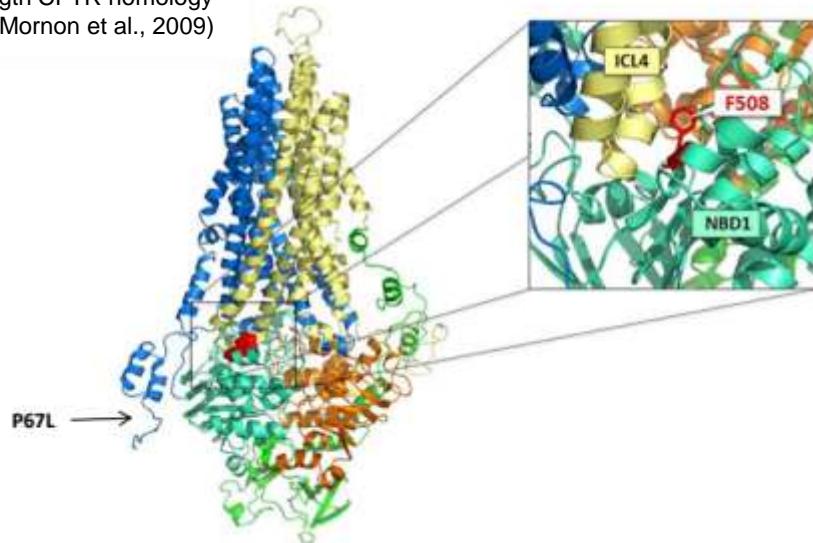
Maturation (12.5%),  
Cl- conductance (10.7%)

## COMPLEX GENOTYPE

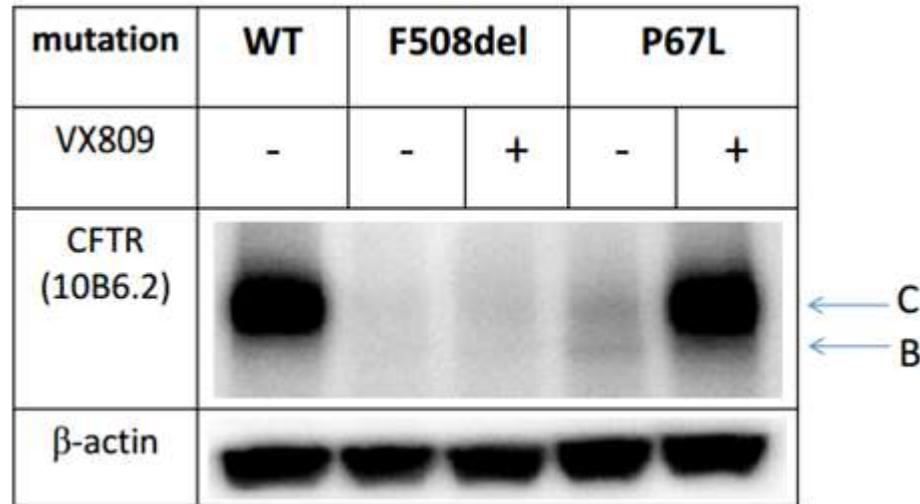
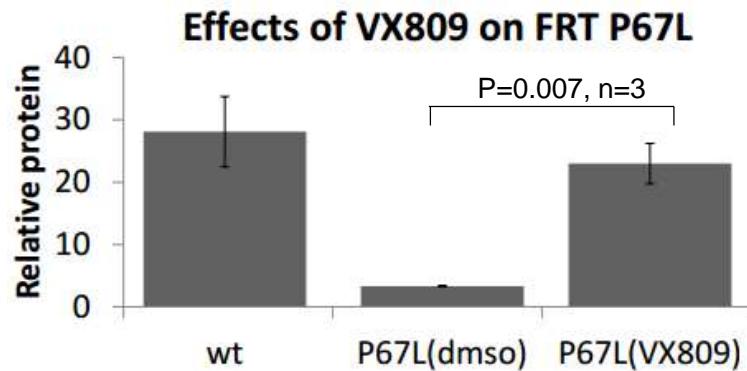
M470 (97%) , V470(3%)

# P67L

Full-length CFTR homology model (Mornon et al., 2009)

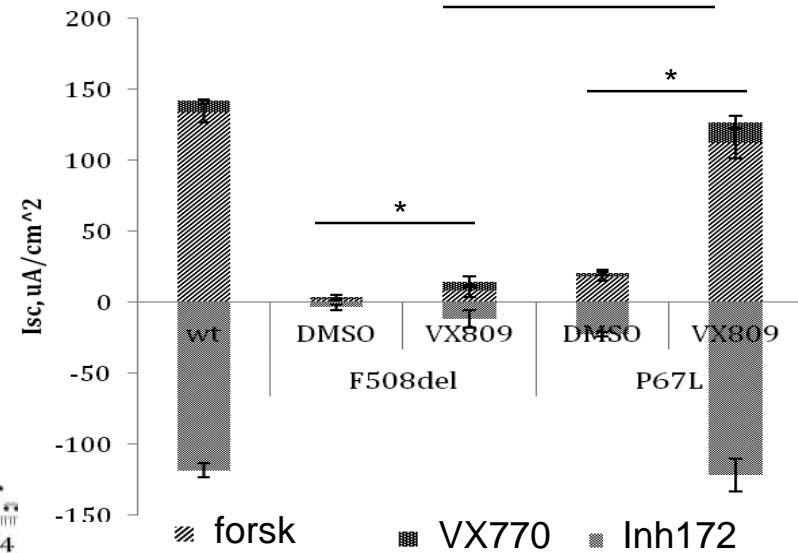
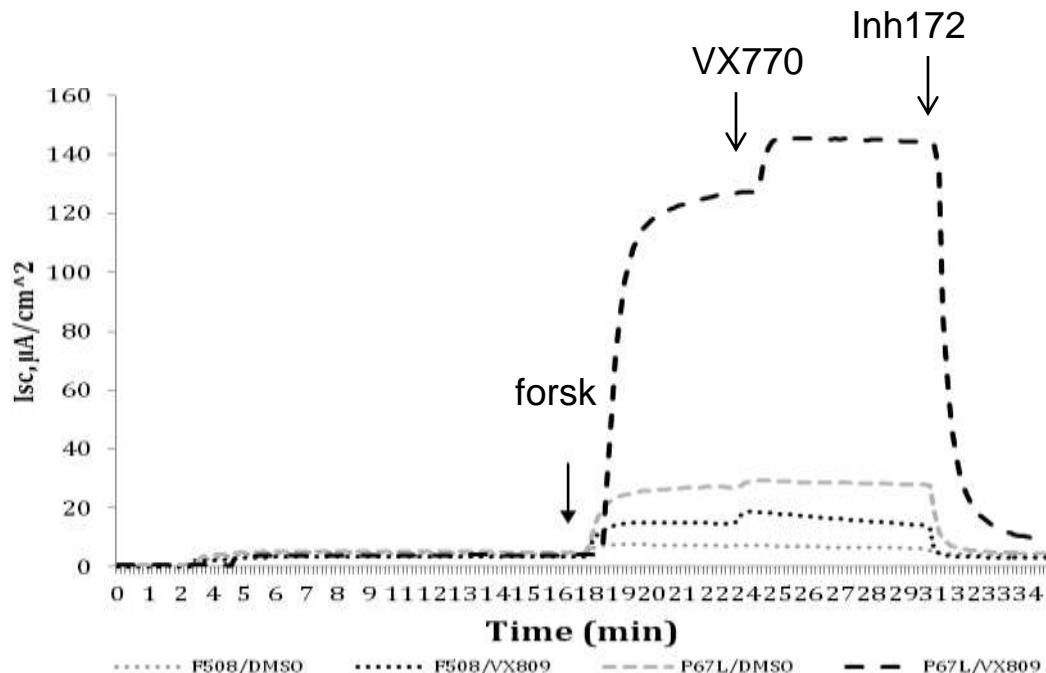


# Lumacaftor-based correction of P67L is robust



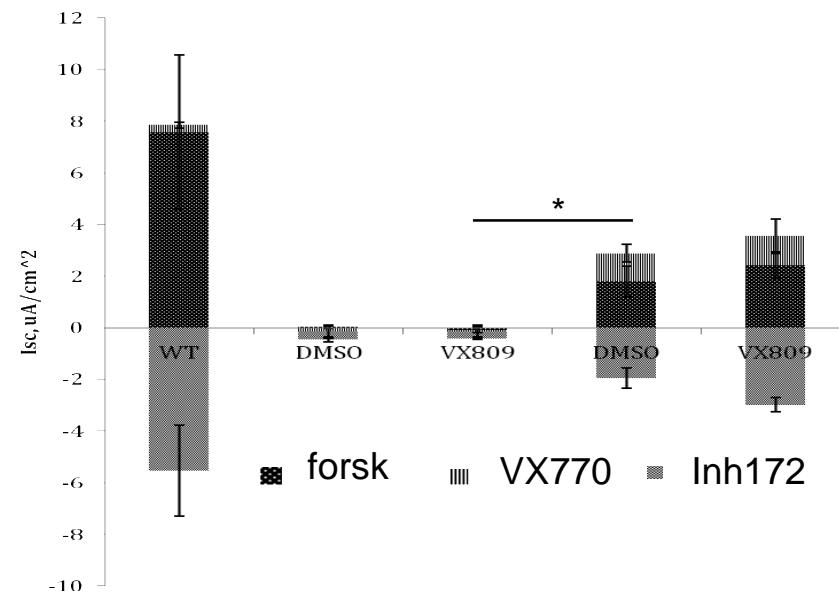
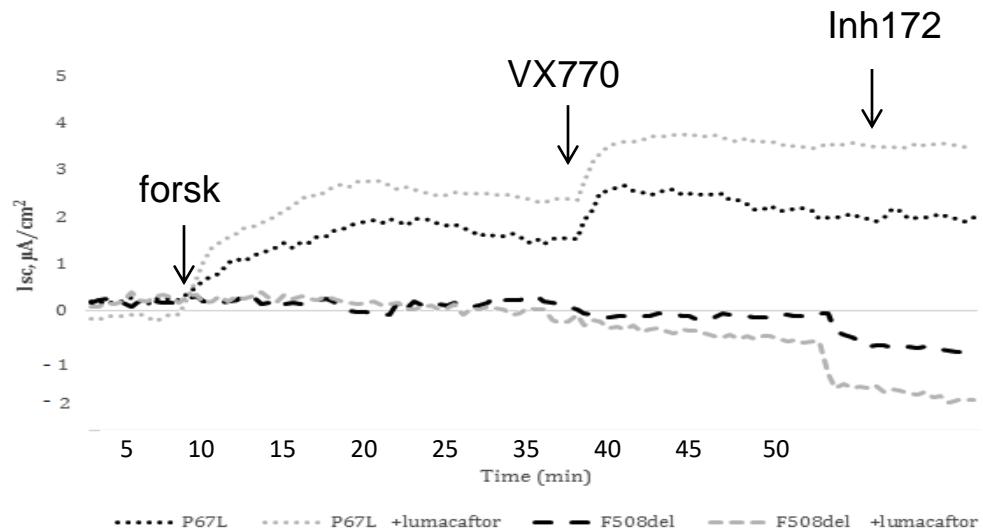
3 uM VX809, FRT cells  
C.Sabusap

# Effects of Lumacaftor (VX809) on P67L CFTR Function



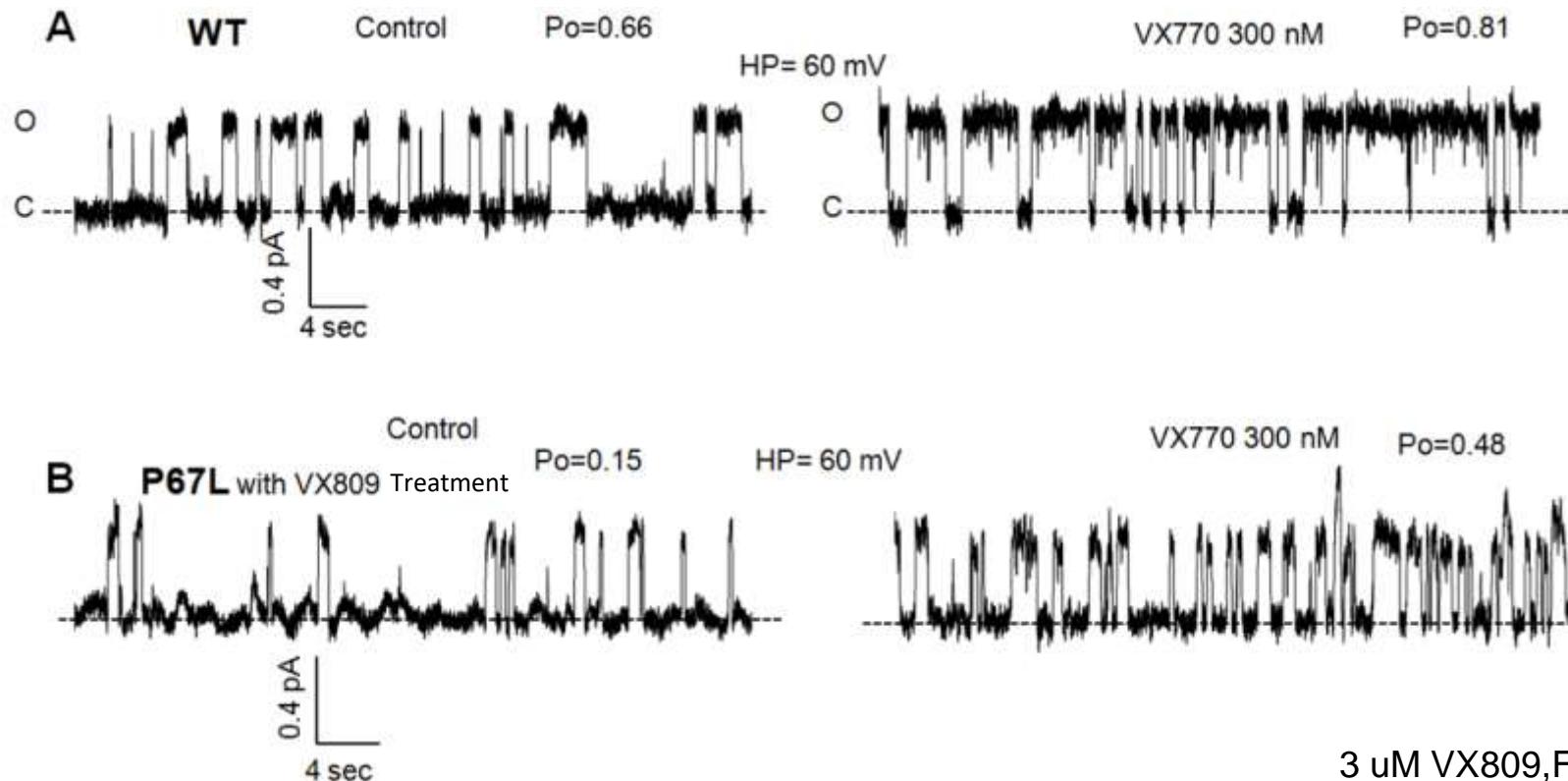
3 uM VX809  
FRT Cells  
J. Hong, n $\geq$ 3  
 $*P<0.05$

# Effects of Lumacaftor (VX809) on P67L CFTR Function in Primary Cells

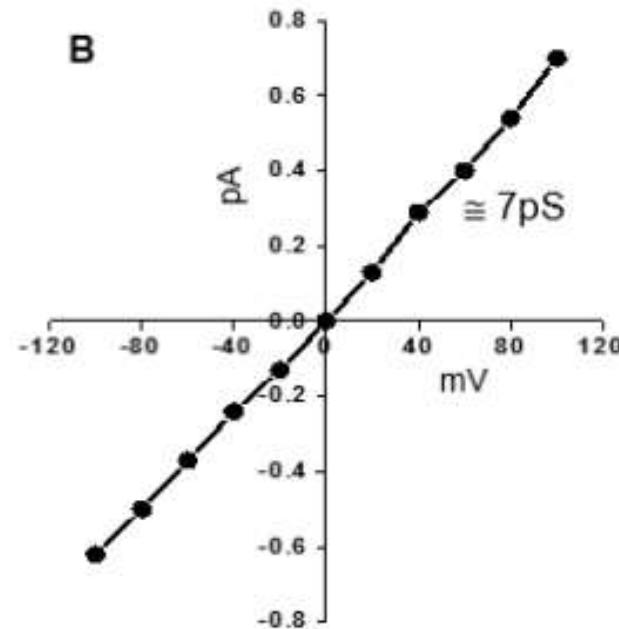
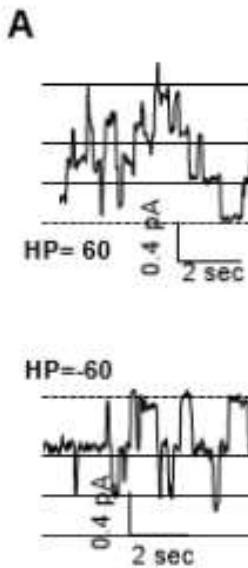


3 uM VX809  
Primary nasal epithelial cells  
C. Sabusap, n≥3  
 $*P<0.05$

# Effects of Modulators on P67L CFTR Open Probability

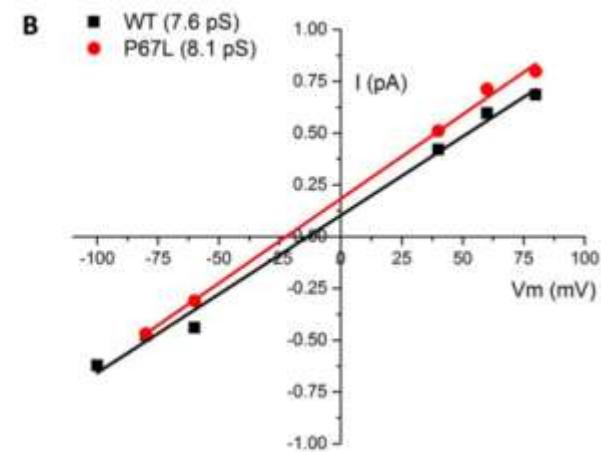


# Lumacaftor and P67L CFTR I/V Relationship



3 uM VX809, FRT cells  
W. Wang

# F508del/P67L Primary Nasal Epithelial Cell Single Channel Analysis



# Summary

- Lumacaftor together with VX770 activates ion transport in cell lines expressing P67L. In primary airway epithelial monolayers, high level constitutive P67L CFTR function is observed, consistent with a recent case report (Chest, Vol 147, No.3, 2015).
- Lumacaftor dramatically increases the amount of fully-glycosylated, mature (band C) P67L detected biochemically.
- Although sometimes designated as a class IV conductance defect, little evidence of an intrinsic conductance abnormality was observed in FRT or primary airway epithelial cells encoding P67L.
- These findings establish the need to reclassify P67L as a processing defect- in addition to the suggestion of gating abnormalities.
- Rare mutations such as P67L (noted in only 239 alleles worldwide by the CFTR2 database) represent a useful model for evaluating challenges to precision medicine among ultra-orphan indications, and highlight shortcomings of the traditional basic science classification scheme.

# Implications

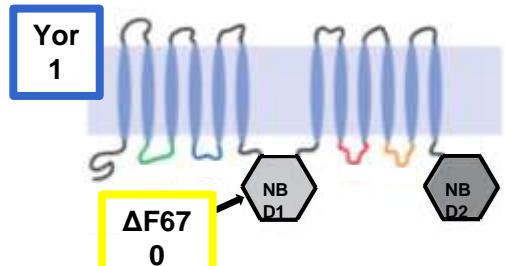
Access to new personalized treatments among patients with ultra-orphan genotypes has been complicated due to challenges arranging Phase III clinical testing, and off-label prescribing limited by failure of third party reimbursement. Rare CFTR mutations such as P67L are emblematic of the challenges to ‘precision’ medicine, including use of the best available mechanistic knowledge to treat patients with unusual forms of disease.

Domain	Folding defect		Legacy Name	TMD1(80-389)		NBD1(390-673)				R domain (674-829)		TMD2 (830-1200)		NBD2 (1201+)	
N-terminal (1-79)	No defect			No defect (4)	7 R334W	Mild (11)	No defect (2)	34 S549N		No folding defect (6)	No defect			91 I1234V	
	No defect				8 T338I		Mild (3)	35 G551D			Mild			92 G1244E	
	Mild (3)	1 E56K			9 R347H			36 S549R			Severe - nonsense mutations (4)	63 K710X		93 S1251N	
		2 P67L			10 R352Q			37 D579G				64 L732X		94 D1270N	
		3 R74W			11 D110H			38 D614G				65 R764X			
	Severe			Mild (11)	12 R117C			39 A455E				66 E822X			
	Nonsense Mutations (3)	4 Q39X			13 R117H			40 L467P				67 L927P			
		5 E60X			14 G178R			41 L470P				68 G970R			
		6 R75X			15 H192G			42 G480C				69 S977F			
	Severe (7)	16 V232D			17 F311del			43 S492F				70 F1052V			
		18 I336K			19 S341P			44 I506T				71 G1069R			
		20 R347P			21 Q359K/T360K			45 I507del				72 D1152H			
		22 G85E			23 G91R			46 F508del				73 S945L			
		24 E92K			25 H199Y			47 V520F				74 R1070Q			
		26 P205S			27 L206W			48 L558S				75 R1070W			
		28 L227R			29 E92X			49 A559T				76 L1065P			
		30 Q98X			31 Y122X			50 R560K				77 R1066C			
		32 Q220X			33 G330X			51 R560T				78 R1066H			
	Nonsense Mutations (5)	34 W401X		Nonsense Mutations (10)	35 S466X			52 Y569D				79 L1077P			
		36 S489X			37 Q493X			53 Q525X				80 M1101K			
		38 Q525X			39 G542X			54 E831X				81 E831X			
		40 R553X			41 G542X			55 W846X				82 W846X			
		42 Q552X			43 R553X			56 R851X				83 R851X			
		44 E585X			45 Q552X			57 E1104X				84 Q890X			
		46 R1158X			47 E1104X			58 W1089X				85 E1104X			
		48 R1162X			49 W1089X			59 Y1092X				86 W1089X			
		50 S1196X			51 Y1092X			60 R1158X				87 R1158X			
		52 S1196X			53 E1104X			61 Y1092X				88 W1282X			
	Severe (1)	54 W1204X		Nonsense mutations (3)	55 R1158X			62 R1162X				89 Q1313X			
		56 W1282X			57 W1282X			63 S1196X				90 Q1313X			
		58 Q1313X			59 Q1313X										
		60 Q1313X			61 Q1313X										

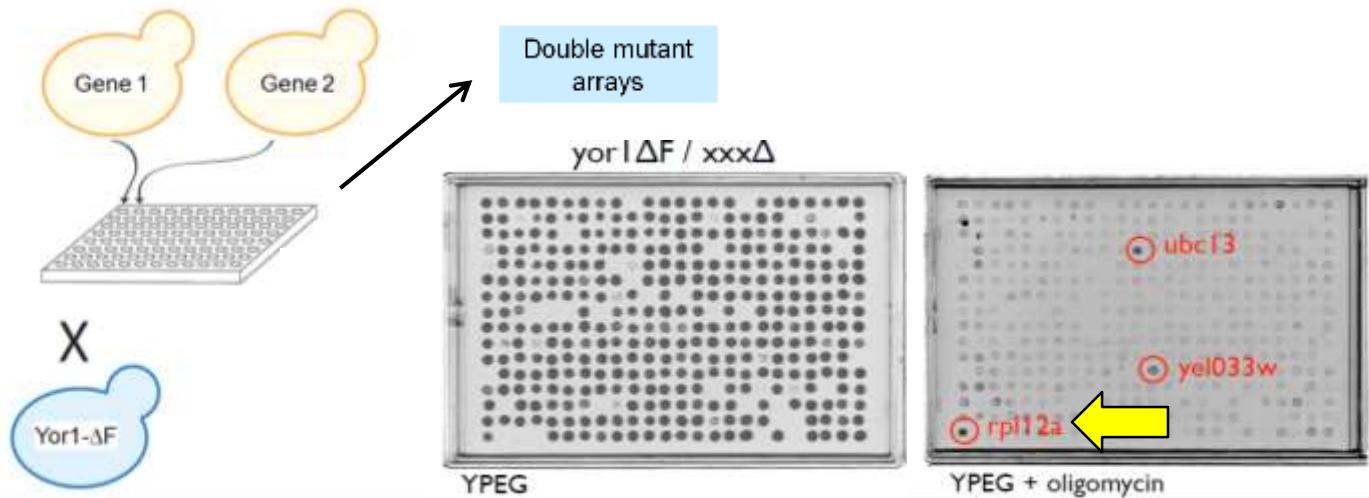
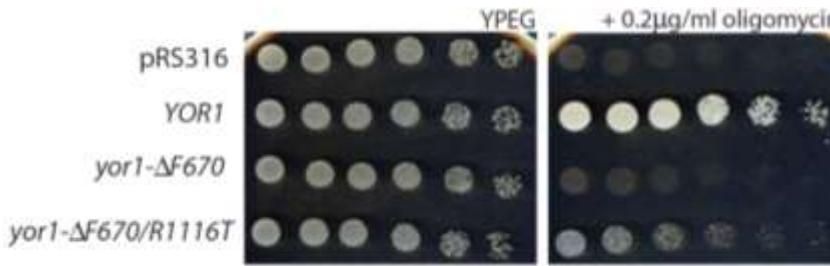
# **Ribosomal Stalk Protein Silencing Rescues the Functional Expression Defect of Mutant CFTR**

---Collaboration with Hartman and Lukacs Laboratories

# High-Throughput Phenomic Screen using Yeast Homolog to CFTR to Identify Genetic Modifiers of F508del



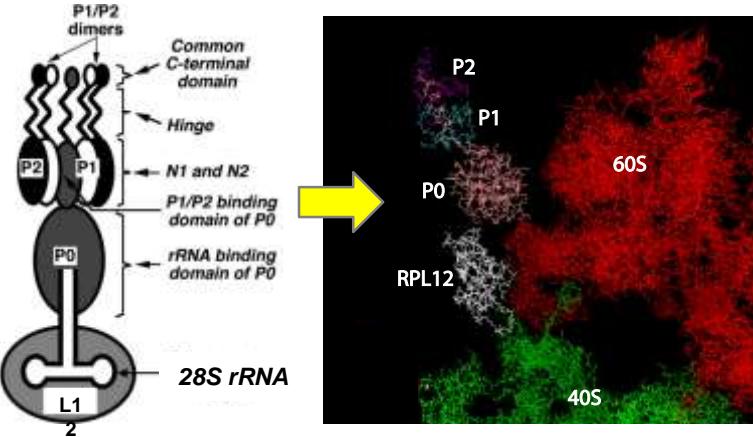
Pagant et al., J Biol Chem 283:26444, 2008.



Louie, Guo, Hartman IV et al. Genome Medicine 4:103, 2012.

# Rpl12

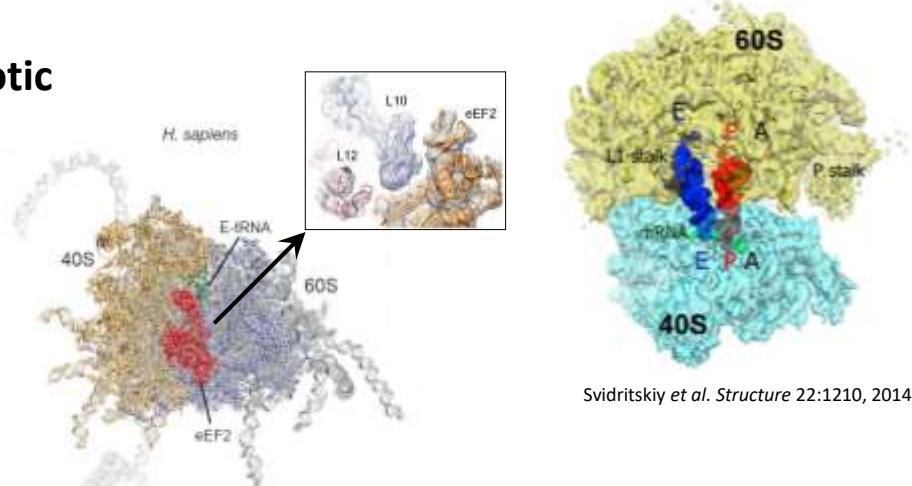
- Ribosomal Protein L12
  - Forms base of the 60S P stalk by directly binding to 28S rRNA



Gonzalo & Reboud. *Biol Cell* 95:179, 2003.

- 60S P stalk interacts with several eukaryotic elongation factors (eEFs)

- Rplp1 and Rplp2 with eEF1 $\alpha$
- Rpl12 with eEF2

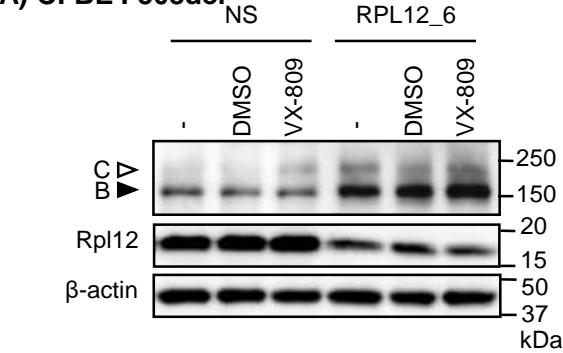


Svidritskiy et al. *Structure* 22:1210, 2014.

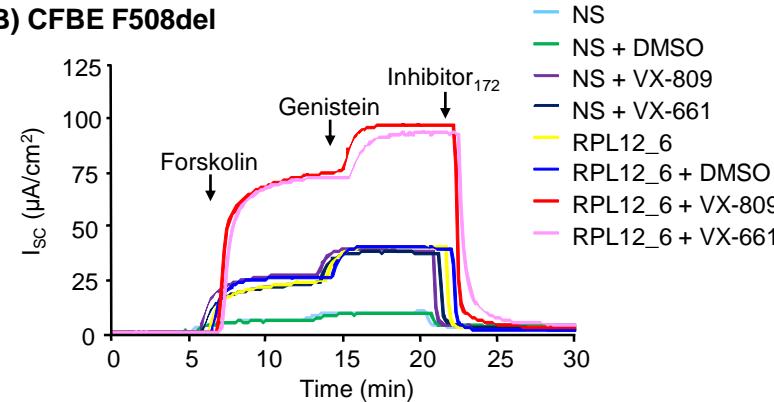
Anger, Beckmann et al. *Nature* 497:80, 2013.

# siRNA-Mediated Knockdown of Rpl12 Corrects Functional Expression of Human F508del CFTR

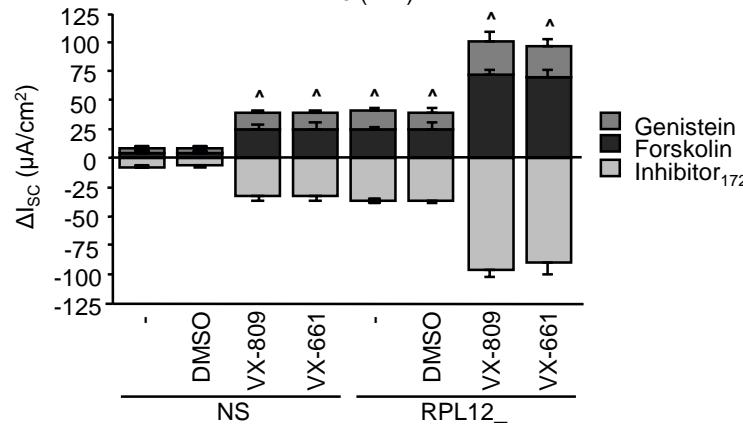
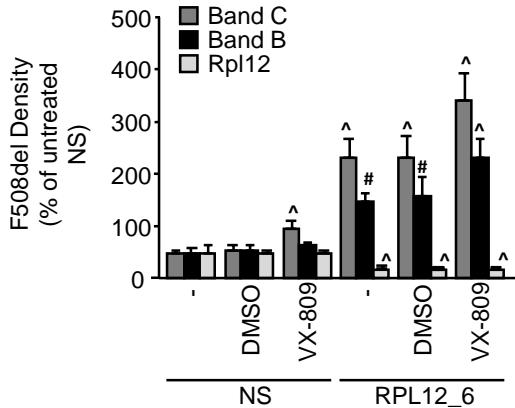
(A) CFBE F508del



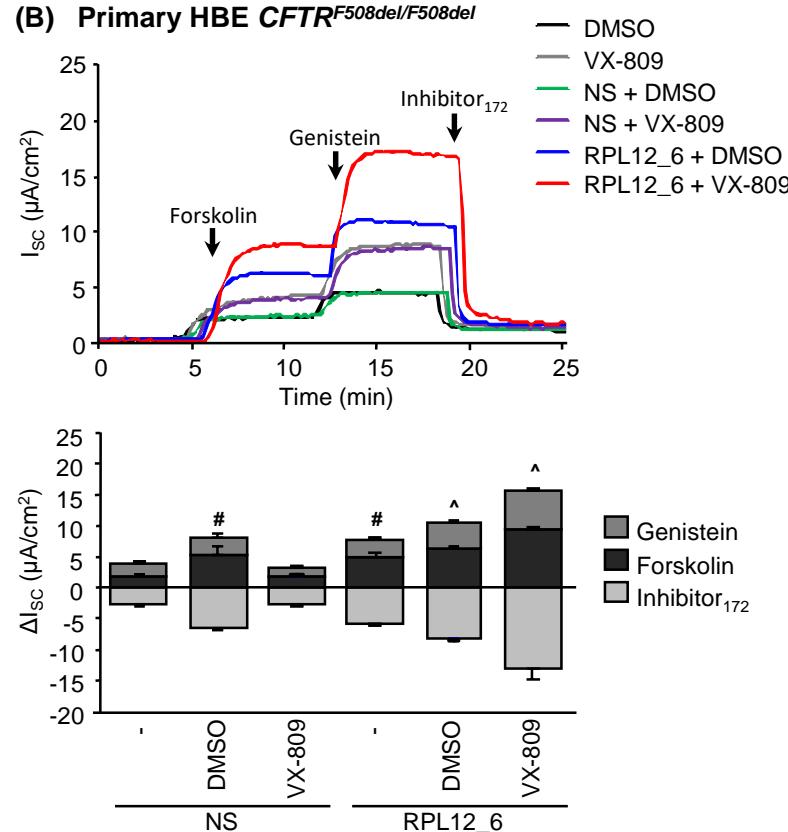
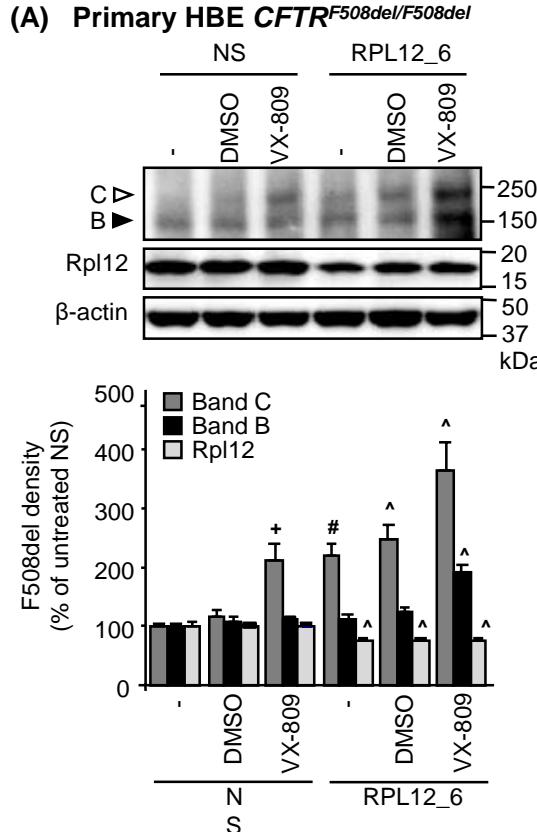
(B) CFBE F508del



$I_{sc}$  (% of untreated NS)



# Rpl12 Suppression Rescues F508del CFTR Maturational Processing in Primary HBE $CFTR^{F508del/F508del}$ Cells



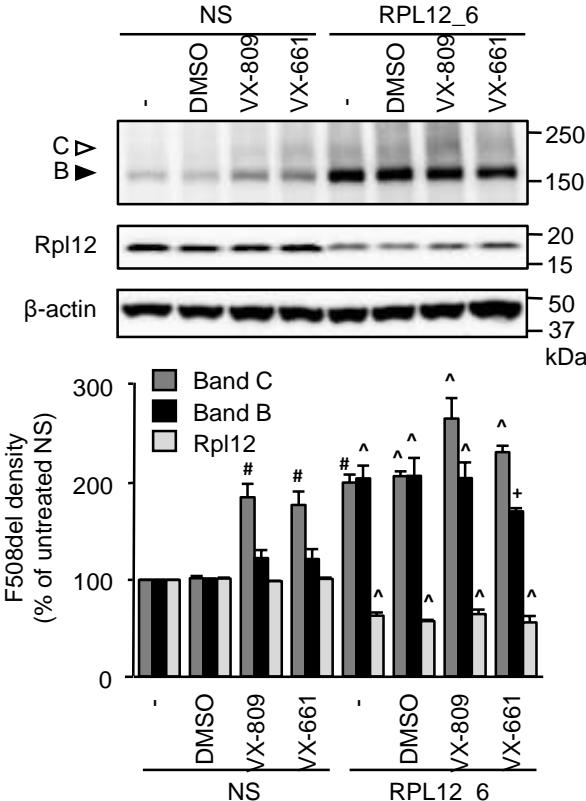
# Previous Mechanistic Studies of F508del Processing and Specificity of the Rpl12 Effect

Collaborating laboratories working on this project have previously shown that:

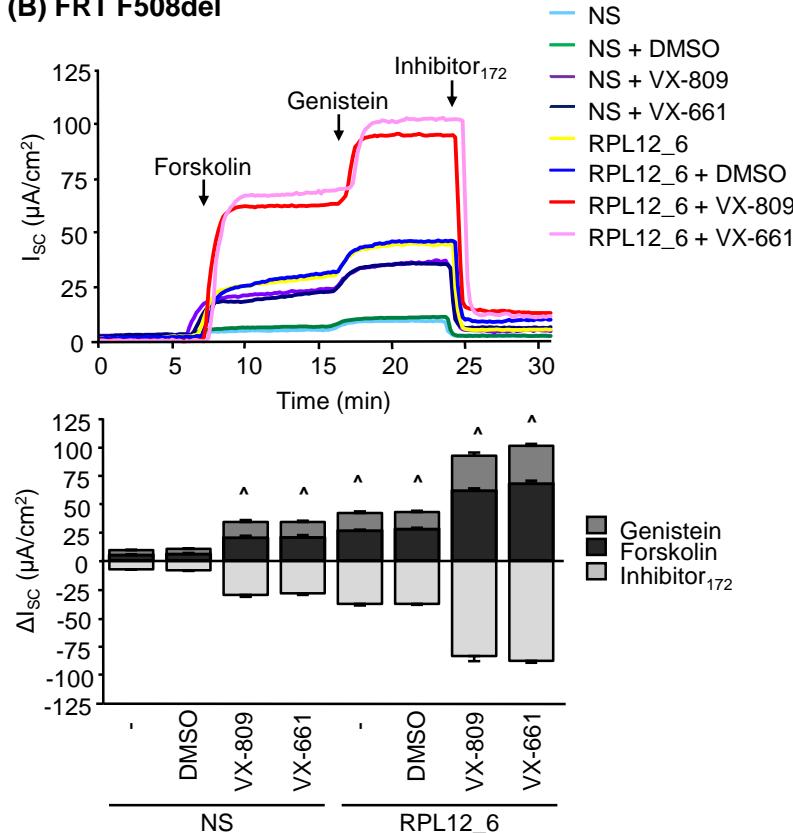
- Rpl12 knockdown increases F508del CFTR protein stability (CHX chase), maturation efficiency (pulse-chase), and PM localization (cell surface ELISA) in CFBE and HeLa cells.
- Rpl12 inhibition does not affect WT CFTR expression (western blot) or ion transport (short circuit current measurements) in CFBE and primary HBE cells.
- Expression patterns of CFTR-associated chaperones (HSP90A, HSPA8, AHA1, BAG1, DNAJA1, STIP1) are unaffected by Rpl12 inhibition (western blot).
- Rpl12 knockdown does not exert global effects on trafficking of other native membrane-associated proteins, nor their mutant counterparts (Tfr, V2R-Y128S, MLC1-P92S, MLC-S280L) with ER retention phenotypes similar to F508del CFTR (cell surface ELISA).

# Previous Findings in HeLa, CFBE and Primary HBE Confirmed in FRT Cells Stably Expressing F508del CFTR

(A) FRT F508del



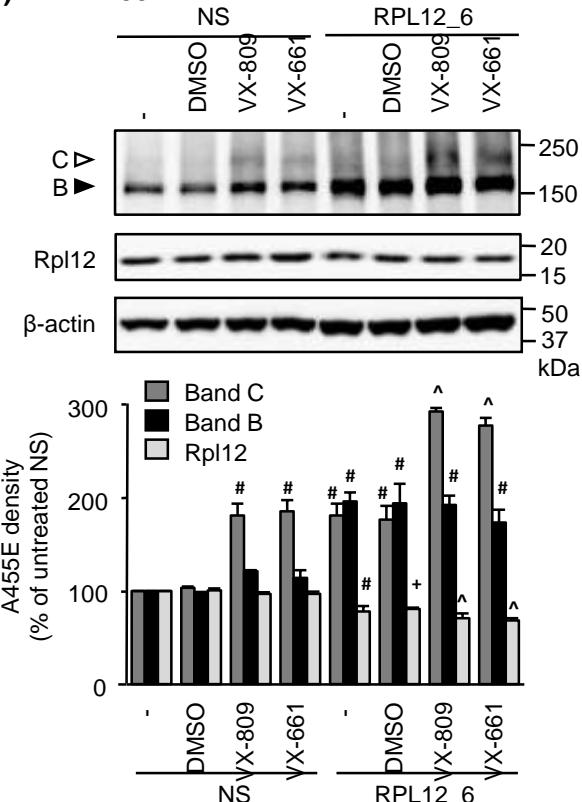
(B) FRT F508del



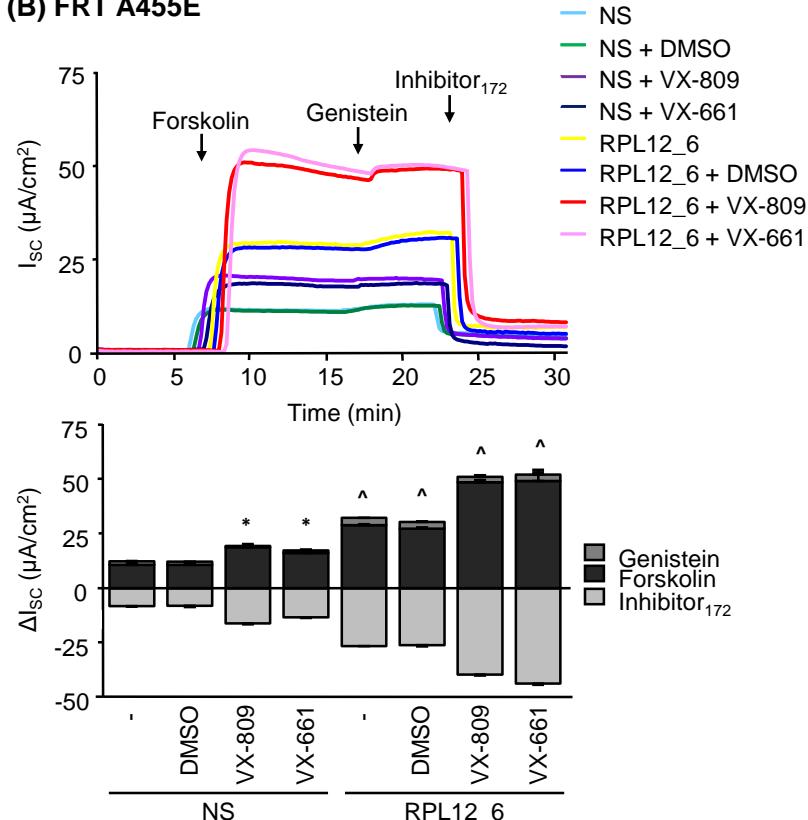
n = 2 each; + < 0.01, # < 0.001, ^ < 0.0001

# Similar to F508del, Functional Expression of A455E CFTR is Rescued by Rpl12 Knockdown

(A) FRT A455E



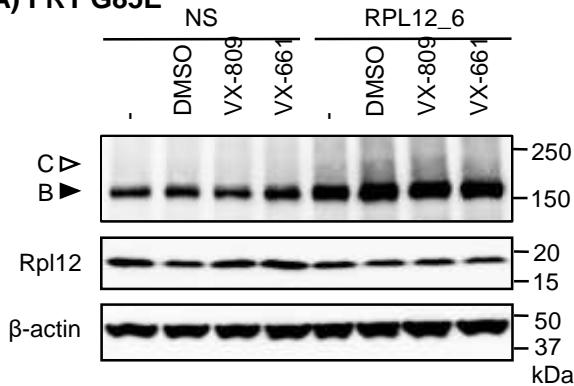
(B) FRT A455E



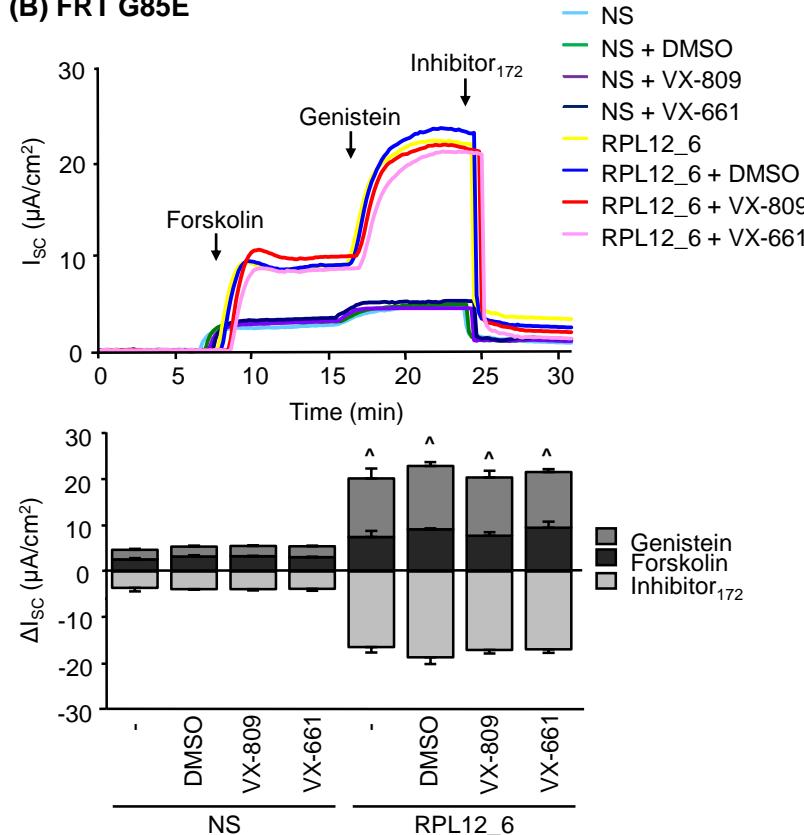
n = 2 each; \* < 0.05, + < 0.01, # < 0.001, ^ < 0.0001

# Rpl12 Suppression Significantly Increases G85E CFTR Processing

(A) FRT G85E

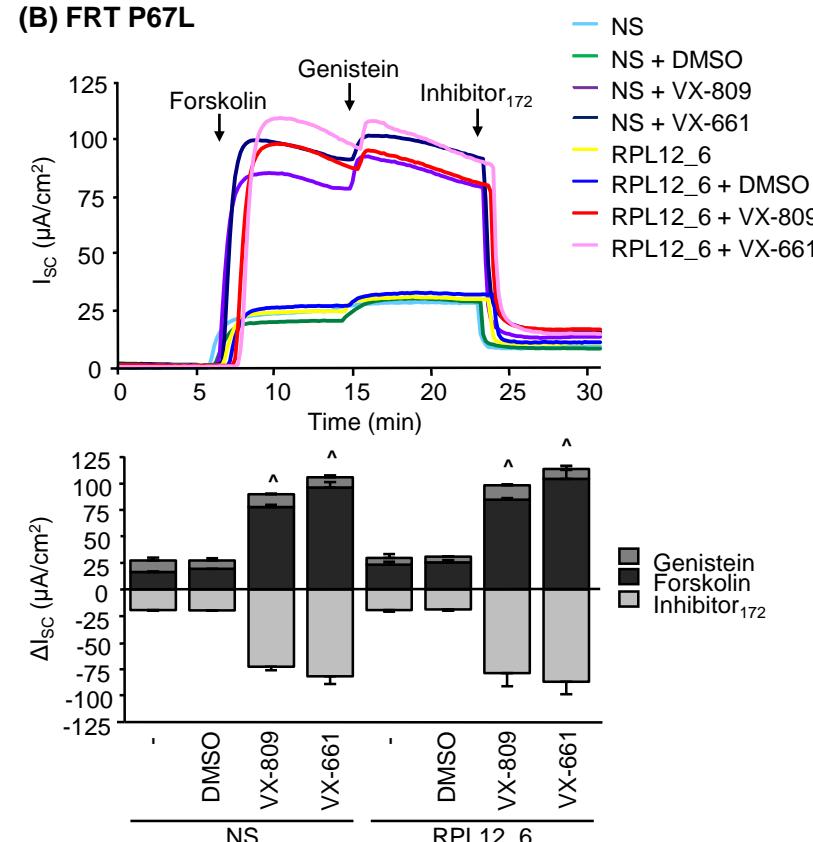
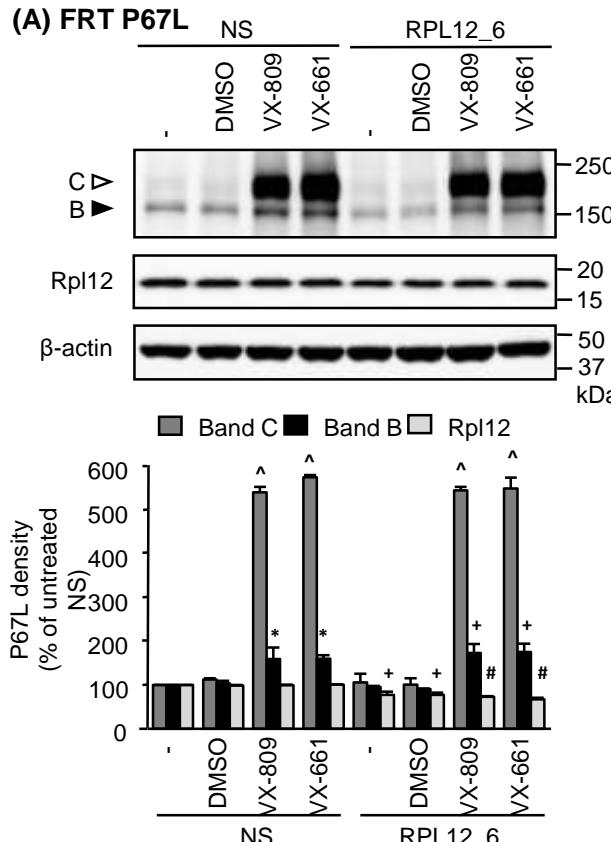


(B) FRT G85E



n = 2 each; + < 0.01, # < 0.001, ^ < 0.0001

# Rpl12 Knockdown does not Rescue P67L CFTR Functional Expression



# *RPL12* Heterozygous Mice are Healthy

## Current Status:

- Three male *RPL12<sup>+/−</sup>* mice are breeding to generate *RPL12<sup>+/−</sup>* females for producing knockouts.
  - No abnormalities observed to date for *RPL12* heterozygosity.
- A number of conditional *RPL12* knockouts are also being generated, including animals with *RPL12* disruption only in the intestinal tract.



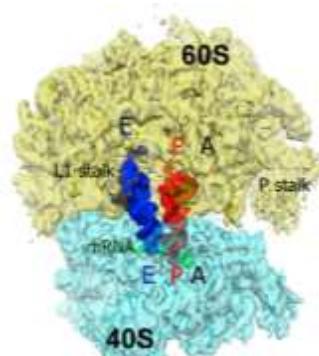
## Future Directions:

- Cross-breed *RPL12<sup>+/−</sup>* and/or *RPL12<sup>−/−</sup>* mouse to *CFTR<sup>F508del</sup>* murine model generated using CRISPR/Cas9 by our collaborator, Dr. Mitchell Drumm (Case Western Reserve University).
- Assay for improvements in F508del processing in the intestine and trachea by *ex vivo* ion transport analysis (short circuit current), and *in vivo* bioelectric measurement (NPD). Also investigate molecular phenotype by biochemical methods (qRT-PCR, western blot, IHC).

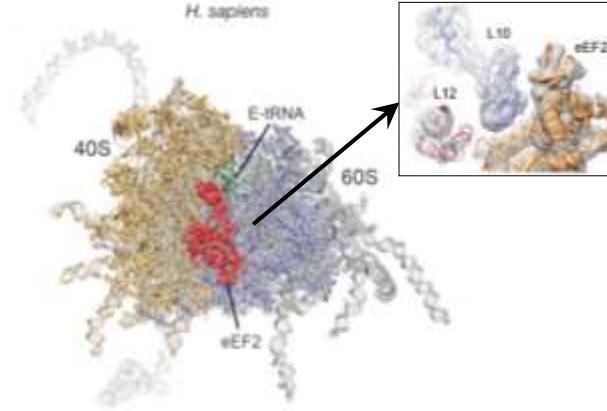
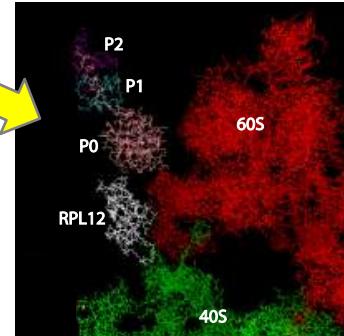


# Summary

- Discovery of the Rpl12 effect on F508del CFTR derives from yeast phenomic analysis. Systems level investigation is ongoing in the Yor1-ΔF670 model of CF and for X-mutations.
- ~50% knockdown of human Rpl12 leads to a robust increase in F508del CFTR maturation, half-life, PM density, and channel function, as well as additivity with small molecule correctors.
- Other CFTR Class II mutations are variably improved by Rpl12 knockdown.
- We hypothesize Rpl12 inhibition slows translation to allow rescue of the F508del CFTR folding defect, which will be investigated by ribosome profiling studies currently under way.
- Rpl12 haplosufficiency in vivo has been demonstrated, and phenotypic studies are in progress.



Svidritskiy *et al.* *Structure* 22:1210, 2014.



Anger, Beckmann *et al.* *Nature* 497:80, 2013.

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