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F1099L-CFTR (c.3297C>G) has Impaired Channel Function and Associates with Mild Disease Phenotypes in Two Pediatric Patients

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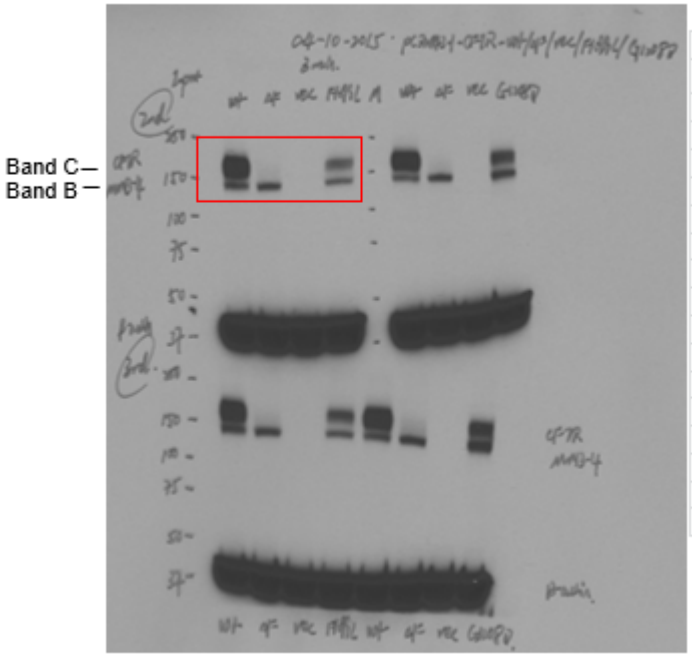
Supplementary Materials

Note:

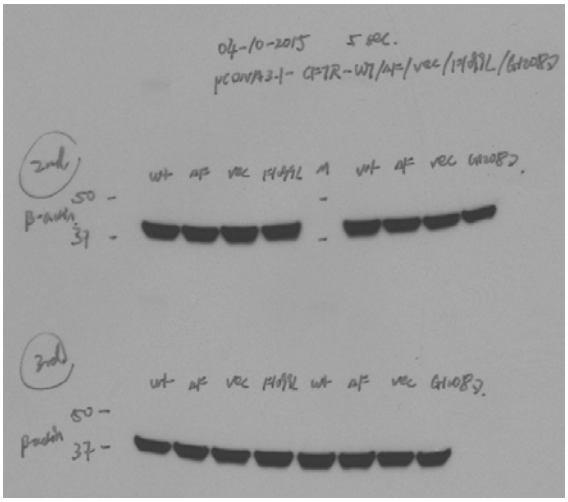
The densities of Western blots bands were quantified using ImageJ software (U.S. National Institutes of Health). We followed the method of 'Analyzing gels and western blots with ImageJ' (https://lukemiller.org/index.php/2010/11/analyzing-gels-and-western-blots-with-image-j/).

The CFTR maturation efficiency was defined as a ratio of the mature form CFTR (density of Band C) to the total CFTR protein (density of Band B plus Band C).

The CFTR total protein level was defined as the density of Band B plus Band C. Prior to comparisons, the CFTR bands densities were first normalized to their respective β-actin levels (bands densities).



(a)

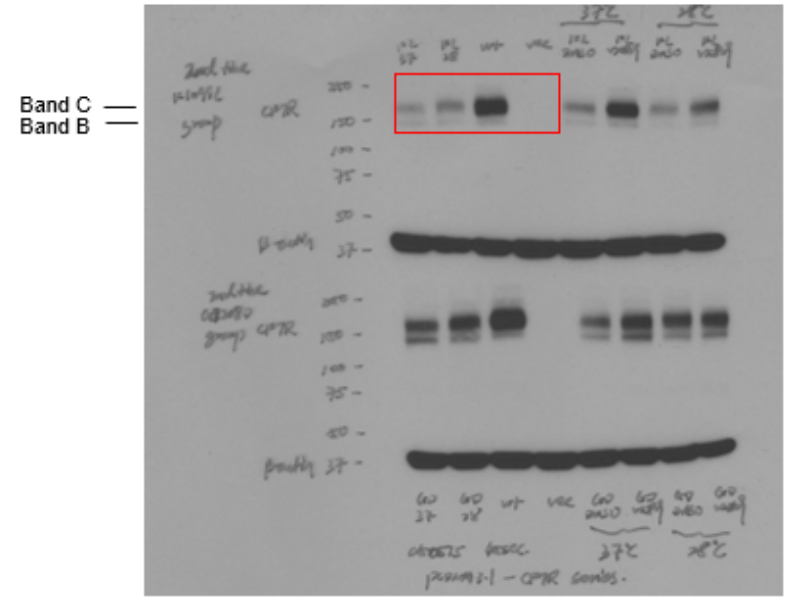


(b)

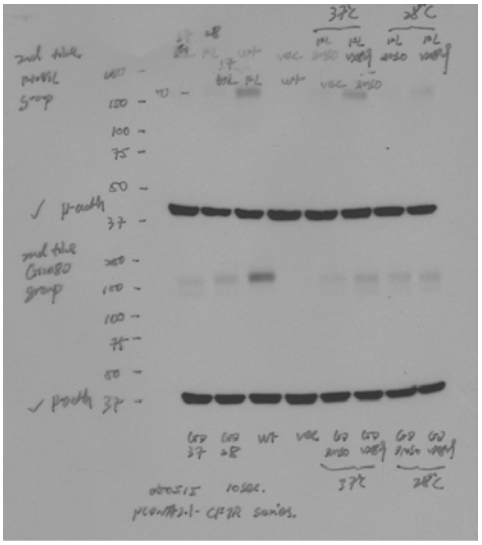
				2nd Exp_04-10-2015									
	1. Band c/ total (band b + c)												
				Raw data (Area)									
					total (Area)		band c/total		band c /total protein (%)				
		WT band C		55940.37			0.863074		86.3074				
		WT band B		8874.898	64815.27								
		F1099L band C		28736.15									
		F1099L band B		16317.58	45053.74		0.63782		63.78195				
	2. Total protein			Using the 'straight line' tool to cover the whole area.									
				Raw data		Ajusted (protein/actin)							
	WT			127205.5		1.361107							
	WT_actin			93457.34									
	F1099L			67753.56									
	F1099L_actin			100471.3		0.674358							
	F1099L/WT (normalized to actin)					0.495448							

(c)

Figure S1. Original Western blot images of Figure 1A in the manuscript. **(a)** (In the red Text Box) from left to right: WT-CFTR, F508del-CFTR, Vector, and F1099L-CFTR. Exposure time: 3 min. The Western blotting experiments were repeated 3 times. Shown in this blot were the 2nd and the 3rd experiments. The blot from the 2nd experiment (in the red Text Box) was used as the representative blot in the manuscript (Figure 1A). **(b)** Original image of β-actin exposed for 5 s. The blot in the red Text Box was used in Figure 1A. **(c)** Densitometry readings of WT-CFTR and F1099L-CFTR bands using ImageJ software. Maturation efficiency (Band c/total (band b+c): WT-CFTR (86.3%), F1099L-CFTR (63.8%). Total protein (F1099L was 49.5% of WT-CFTR).



(a)

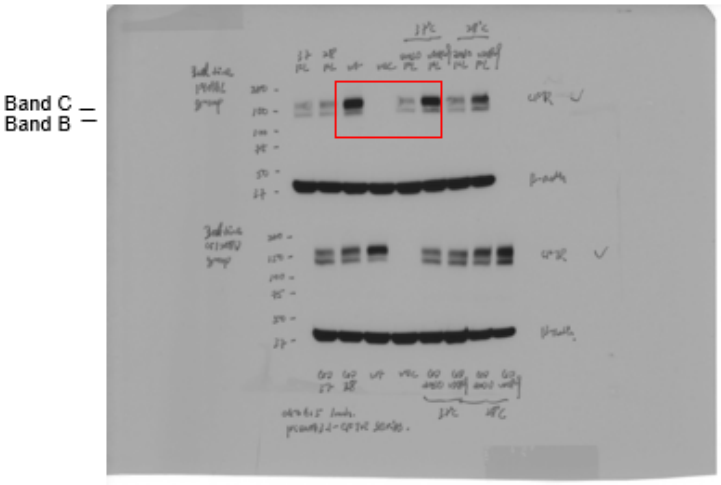


(b)

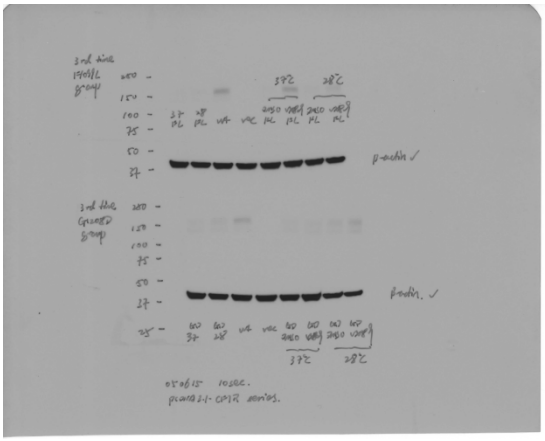
	1. Band c/ total (band b + c)			Note: using method option 2										
			Raw data (Area)											
				total (Area)		**band c/total**		**band c /total protein (%)**			**fold change**		**% of WT**	
	Temp sensitivity													
	37 C_Band C		12782.47											
	37 C_Band B		2618.175	15400.65		0.829996		82.99958			1		83.62095	
28 C	**Band C**		23597.24											
	Band B		1599.447	25196.69		0.936522		93.65215			1.128345		94.35328	
WT_37	**Band C**		61725.28											
	Band B		462.104	62187.39		0.992569		**99.25692**					**100**	

(c)

Figure S2. Original Western blot images of Figure 2A in the manuscript. **(a)** (In the red Text Box) from left to right: F1099L-CFTR (37 °C), F1099L-CFTR (28 °C), WT-CFTR, and Vector. The Western blotting experiments were repeated 3 times. Shown in this blot was the 2nd experiment. The bands in the red Text Box were used as the representative blot in the manuscript (Figure 2A). Exposure time: 45 s. **(b)** Original image of β-actin exposed for 10 sec. The blot in the red Text Box was used in Figure 2A. **(c)** Densitometry readings of F1099L-CFTR bands at different temperature using ImageJ software. Fold change in maturation efficiency (1.13-fold at 28 °C compared to at 37 °C).



(a)



(b)

				Exp.3_05-06-2015									
	1. Band c/ total (band b + c)			Note: using method option 2									
			Raw data (Area)										
				total (Area)		**band c/total**		**band c /total protein (%)**			**fold change**		**% of WT**
	Temp sensitivity												
	37 C_Band C		7894.702										
	37 C_Band B		3539.317	11434.02		0.690457		69.04573	(control)		1		72.72374
28 C	**Band C**		12444.77										
	Band B		3145.832	15590.6		0.798222		79.82225			1.156078		84.07431
WT_37	**Band C**		52993.43										
	Band B		2822.915	55816.34		0.949425		94.94249					**100**
	VX-809 effect												
DMSO	**37 C_Band C**		10107.09										
	37 C_Band B		3142.539	13249.63		0.762821		76.28206			1		80.34554
VX-809	**37 C_Band C**		50233.39										
	37 C_Band B		4683.472	54916.86		0.914717		91.4717			1.199125		96.34432
DMSO	**28 C_Band C**		14564.91										
	28 C_Band B		2905.953	17470.86		0.833669		83.36686			1.092876		87.80774
VX-809	**28 C_Band C**		30135.22										
	28 C_Band B		6317.007	36452.23		0.826705		82.67045			1.083747		87.07424
	2. Total protein (Band B + Band C)												
	Temp sensitivity												
				Raw data		**Ajusted (protein/actin)**			**Fold change**		**% of WT**		
	37 C_Band C			**11434.02**									
	37 C_Band B		Actin	58588.06		0.19516	(control)		1		20.55954		
28 C	**Band C**			15590.6									
	Band B		Actin	55389.87		0.28147			1.442257		29.65214		
WT_37	**Band C**			55816.34									
	Band B		Actin	58801.04		0.949241					100		
	VX-809 effect												
DMSO	**37 C_Band C**			13249.63									
	37 C_Band B		Actin	61557.23		0.215241	(control)		1		22.67505		
VX-809	**37 C_Band C**			54916.86									
	37 C_Band B		Actin	60061.35		0.914346			4.248015		96.32393		
DMSO	**28 C_Band C**			17470.86									
	28 C_Band B		Actin	56403.18		0.30975			1.439084		32.63129		
VX-809	**28 C_Band C**			36452.23									
	28 C_Band B		Actin	52746.33		0.691086			3.210756		72.80404		

(c)

Figure S3. Original Western blot images of Figure 2C in the manuscript. **(a)** (In red Text Box) from left to right: WT-CFTR, Vector, F1099L-CFTR (with DMSO), F099L-CFTR (VX-809). The Western blotting experiments were repeated 3 times. Shown in this blot was the 3rd experiment. The blot in the red Text Box was used as the representative blot in the manuscript (Figure 2C). Exposure time: 1 min. **(b)** Original image of β-actin exposed for 10 s. The blot in the red Text Box was used in Figure 2C. **(c)** Densitometry readings of F1099L-CFTR bands with VX-809 or DMSO treatment at different temperature using ImageJ software. VX-809 increased the maturation efficiency (1.2-fold) and total protein (4.2-fold) of F1099L-CFTR.

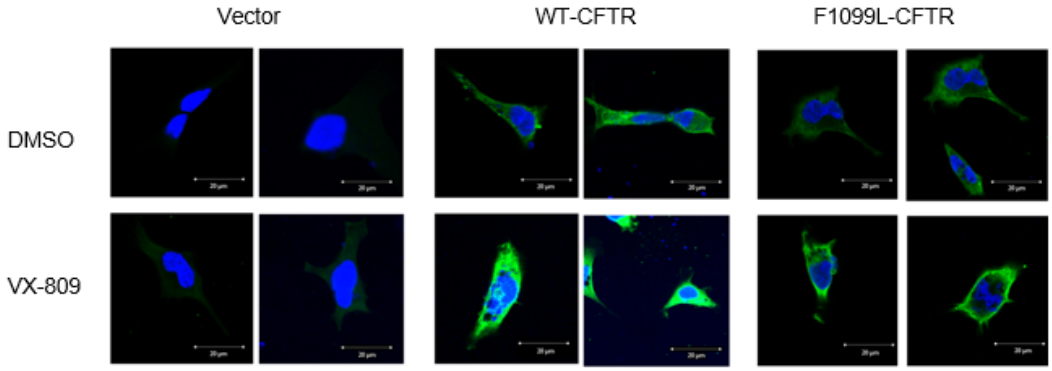


Figure S4. VX-809 increased the total CFTR protein level of F1099L mutation and promoted its maturation as evidenced by immunofluorescence imaging data. F1099L-CFTR-, WT-CFTR-, and vector-transfected HEK-293 cells were treated with VX-809 (5 μM) or DMSO and then subjected to immunofluorescence labeling and imaging.