## SUPPLEMENTAL METHODS

### CFTR outcome measures

NPD was performed by qualified operators based on a Therapeutics Development Network Standard Operating Procedure originally described by Knowles et al., modified for the conduct of a placebo controlled trial.[1] The modified technique included use of an agar nasal probe catheter, electronic data capture (AD Instruments, Colorado City, CO), and interpretation by a single reviewer blinded to treatment assignment, as previously described.[2] Sweat chloride was conducted by pilocarpineiontophoresis using the Macroduct collection device in two separate arm locations for each measurement and analyzed in a central analysis laboratory blinded to treatment assignment.[3] Rectal biopsy to detect fully mature, C-band F508del-CFTR by immunoblot was performed in some of the subjects as an optional exploratory endpoint.

### Pharmacokinetic Analyses

Blood samples for PK analysis were collected predose and at 0.75, 1.5, 3, 4, 6, 9, 12, and 24 hours on Day 1, and pre-dose and at 0.75, 1.5, 3, 4, 6, 9, 12, 24, 30 to 60 and 120 to 180 hours post last dose on Day 28. In addition, predose blood samples were taken on Day 7, Day 14, and Day 21. Pharmacokinetic (PK) analysis was performed using a non-compartmental approach implemented in WinNonlin® 5.1.1 (Pharsight Corporation, Mountain View, USA).

For NPD purposes, a sample size of 21 subjects per dose group was suitable to detect a –4 mV change in chloride-free + isoproterenol with 80% power (within group comparisons).

**Exclusion criteria**

Subjects were excluded if they had a potentially confounding history of illness; ongoing acute illness, including acute respiratory infections; a pulmonary exacerbation or changes in therapy for pulmonary disease within 14 days before the first dose of study drug; liver function tests greater than three times the upper limit of normal; or a history of abnormal renal function in the year prior to enrollment. Subjects were also excluded if they had a change in treatment with intranasal medication or systemic antibiotics in the 14 days prior to study drug dosing; required use of continuous (24 hours per day) supplemental oxygen; or utilized any inhibitors or inducers of cytochrome P450 3A4.

**Rectal biopsy and Western blot**

Up to four rectal biopsies were collected prior to initiation of study drug dosing ("pre-dose") and following 21 or 28 days of VX-809 or placebo ("post-dose") from consented subjects in the trial. The post-dose biopsy procedure was performed 20-24 hours post-oral dosing. The biopsies were frozen on dry ice and stored at -80°C until centralized and batched analysis. Briefly, individual rectal biopsy samples (blinded) were lysed with lysis buffer (200mM Tris, pH 7.5 [ Invitrogen, Cat. #-15567-027], 10nM NaCl [Sigma, Cat. #-5316], 1mM EDTA [Vertex Chemical Supplies], 0.5% sodium deoxycholate [Pierce, Cat. #-89905], 1% NP-40 [Pierce, Cat. #-28324] using a 2mL dounce homogenizer in the presence of protease inhibitors (Complete, EDTA-free protease inhibitors [Roche, Cat. #-5056489001]). Samples were subjected to SDS-PAGE and immunoblot analysis for CLCN2 (mouse anti-CLCN2 antibody, Abnova, Cat. #-H00001181-M01) and GAPDH (mouse anti-GAPDH antibody, Chemicon, Cat. #- mAB374). CLCN2 expression was

used as an indicator of epithelia cell content. A pair of rectal biopsy samples (one pre-dose and one post-dose) were selected for each subject based on CLCN2 expression levels, and further analyzed to determine the presence or absence of CFTR C band. To detect B and C Band CFTR, samples were probed with Riordan-769 anti-CFTR (1:1000 dilution of antibody in TBST/5% milk (5% dry milk powder in Tris buffered saline, pH 7.5 with 10% Tween-20). The total protein load was adjusted for each sample to achieve similar CLCN2 loading between the paired rectal biopsy samples. CFTR standard samples were created by spiking in appropriate amounts of wild-type rectal biopsy protein lysates into a F508del rectal biopsy lysate pool. 10 mcg of each standard were loaded per lane and analyzed in parallel to determine the sensitivity for each blot. Rectal biopsies from normal subjects were obtained from adult patients undergoing clinically indicated sigmoidoscopy/colonoscopy as part of cancer screening or evaluation of lower GI symptoms. IRB approval and informed consent were obtained under a separate protocol at two study sites.

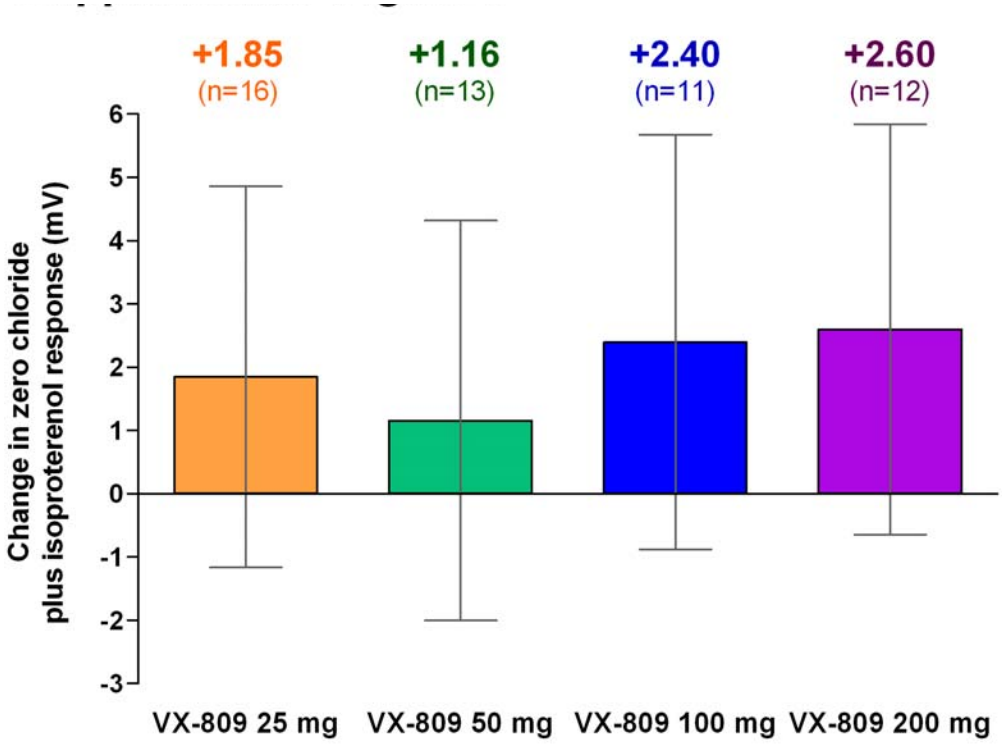
**Supplemental Figure Legends:**

**Supplemental Figure 1: NPD chloride-free plus isoproterenol response change from baseline to Day 28 (mean [95% CI]) treatment difference versus placebo (ANCOVA analysis).** No significant changes in CFTR-dependent chloride transport were seen at 28 days vs baseline or placebo. No differences in CFTR-dependent sodium or chloride transport were seen at days 14 and 28 compared with baseline or placebo for any of the dose groups (data not shown).

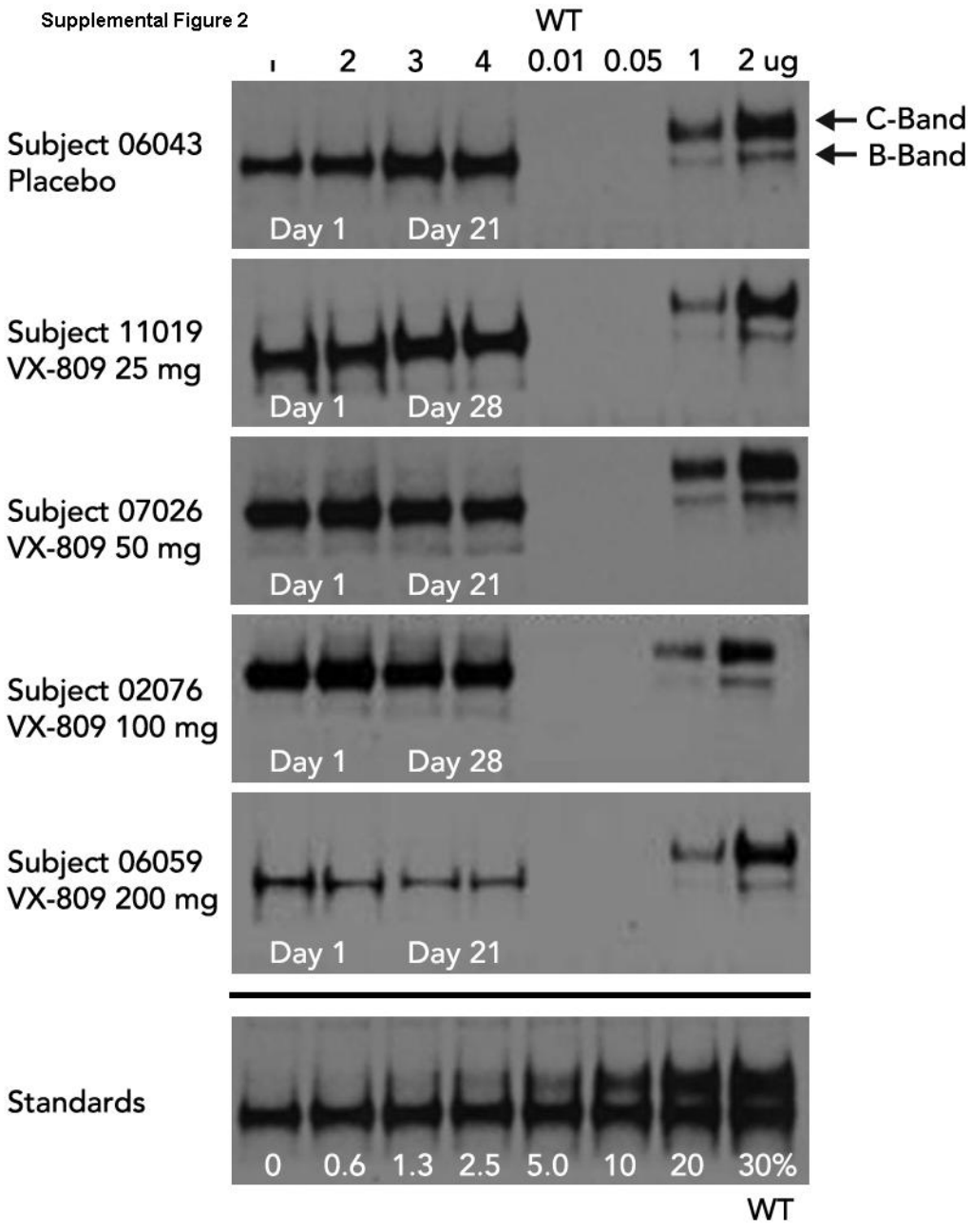
**Supplemental Figure 2:** Examples of immunoblots of rectal biopsies in F508del/F508del study subjects across the five study groups. The left four lanes are from study subjects on day 01, day 21, or day 28 as indicated, and the lanes to the right are spiked samples from subjects without CF (WT). Dilutional rectal biopsy standards from non-CF subjects (WT) are shown at the bottom of the figure ('Standards').

**Supplemental Figures:**

**Supplemental Figure 1:**



Supplemental Figure 2



**Supplemental Table 1: Subject-reported QoL (CFQ-R): Mean change from baseline to Day 28 (ANCOVA analysis).** Although isolated statistically different values were measured for subdomains of the CFQ-R, no clear drug or dose related changes were apparent.

| Domain | VX-809 200 mg (n = 18) | VX-809 100 mg (n = 16) | VX-809 50 mg (n = 17) | VX-809 25 mg (n = 17) | Placebo (n = 17) |
|---|---|---|---|---|---|
| **Body** | 0.06 | 2.61 | -1.63 | -0.21 | -1.34 |
| **Digestion** | 2.58 | 0.25 | -0.72 | 2.28 | 4.62 |
| **Eating** | -2.58 | 3.24 | - 7.27*† | -3.66 | 2.11 |
| **Emotion** | -2.62 | 3.49 | -1.36 | -3.22 | 4.86 |
| **Health perceptions** | -1.9 | -0.44 | - 6.97*† | -2.84 | 5.03 |
| **Physical** | -0.98 | -3.46 | - 7.38* | -5.97 | 1.23 |
| **Respiratory**[α] | 2.22 | -1.29 | - 6.32*† | - 5.22† | 4.53 |
| **Role** | - 6.53*† | 1.1 | -4.6 | - 5.94*† | 2.21 |
| **Social** | -2.64 | 0.47 | -1.01 | 0 | -0.55 |
| **Treatment burden** | -0.68 | 1.42 | - 5.96*† | 4.19 | 2.46 |
| **Vitality** | 0.73 | -1.52 | - 7.23* | -4.65 | -2.18 |
| **Weight** | -4.19 | 8.83 | 2.18 | 5.41 | 0.3 |

[α] MCID in Respiratory domain is improvement ≥4

* $P < 0.05$ within-subject; † $P < 0.05$ versus placebo

## Supplemental References

1 Knowles MR, Paradiso AM, Boucher RC. In vivo nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. Hum Gene Ther 1995; **6**:445-455

2 Solomon GM, Konstan MW, Wilschanski M, et al. An international randomized multicenter comparison of nasal potential difference techniques. Chest 2010; **138**:919-928

3 Accurso FJ, Rowe SM, Clancy JP, et al. Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. N Engl J Med 2010; **363**:1991-2003