SUPPLEMENTAL MATERIAL

Acidic pH Increases Airway Surface Liquid Viscosity in Cystic Fibrosis

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Ratio (Glycan/Gal)	non-CF	CF	P Value
(Neu5Ac+Neu5Gc)/Gal	0.68±0.15	0.75±0.13	0.75
Fuc/Gal	0.62±0.21	0.47±0.13	0.43
$GalNH_2$/Gal	0.52±0.07	0.38±0.05	0.19
$GlcNH_2$/Gal	1.64±0.22	1.40±0.06	0.36
Gal/Gal	1.00	1.00	-
Man/Gal	1.10±0.40	1.04±0.36	0.68

Ratio (Glycan/Man)	non-CF	CF	P Value
(Neu5Ac+Neu5Gc)/Man	1.35±0.63	3.25±2.35	0.34
Fuc/Man	0.97±0.32	0.87±0.33	0.78
$GalNH_2$/Man	1.31±0.58	1.31±0.74	0.99
$GlcNH_2$/Man	3.90±1.68	4.25±2.25	0.75
Gal/Man	3.21±1.64	3.22±1.82	1.00
Man/Man	1.00	1.00	-

Ratio (Glycan/$GalNH_2$)	non-CF	CF	P Value
(Neu5Ac+Neu5Gc)/$GalNH_2$	1.25±0.19	2.13±0.42	0.07
Fuc/$GalNH_2$	1.09±0.26	1.27±0.30	0.46
$GalNH_2$/$GalNH_2$	1.00	1.00	-
$GlcNH_2$/$GalNH_2$	3.24±0.34	4.03±0.72	0.35
Gal/$GalNH_2$	2.07±0.25	2.93±0.58	0.28
Man/$GalNH_2$	1.96±0.73	3.45±1.61	0.23

Table S1. Monosaccharides in non-CF and CF ASL. ASL was removed from non-CF and CF piglets after methacholine administration. Monosaccharides were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD). N=5 per genotype, each from a different pig; littermate controls were used. Data are mean±SEM. P values were by unpaired Student's t-test. Monosaccharides were normalized to galactose (Gal), mannose (Man), and galactosamine ($GalNH_2$) levels.

Table S1

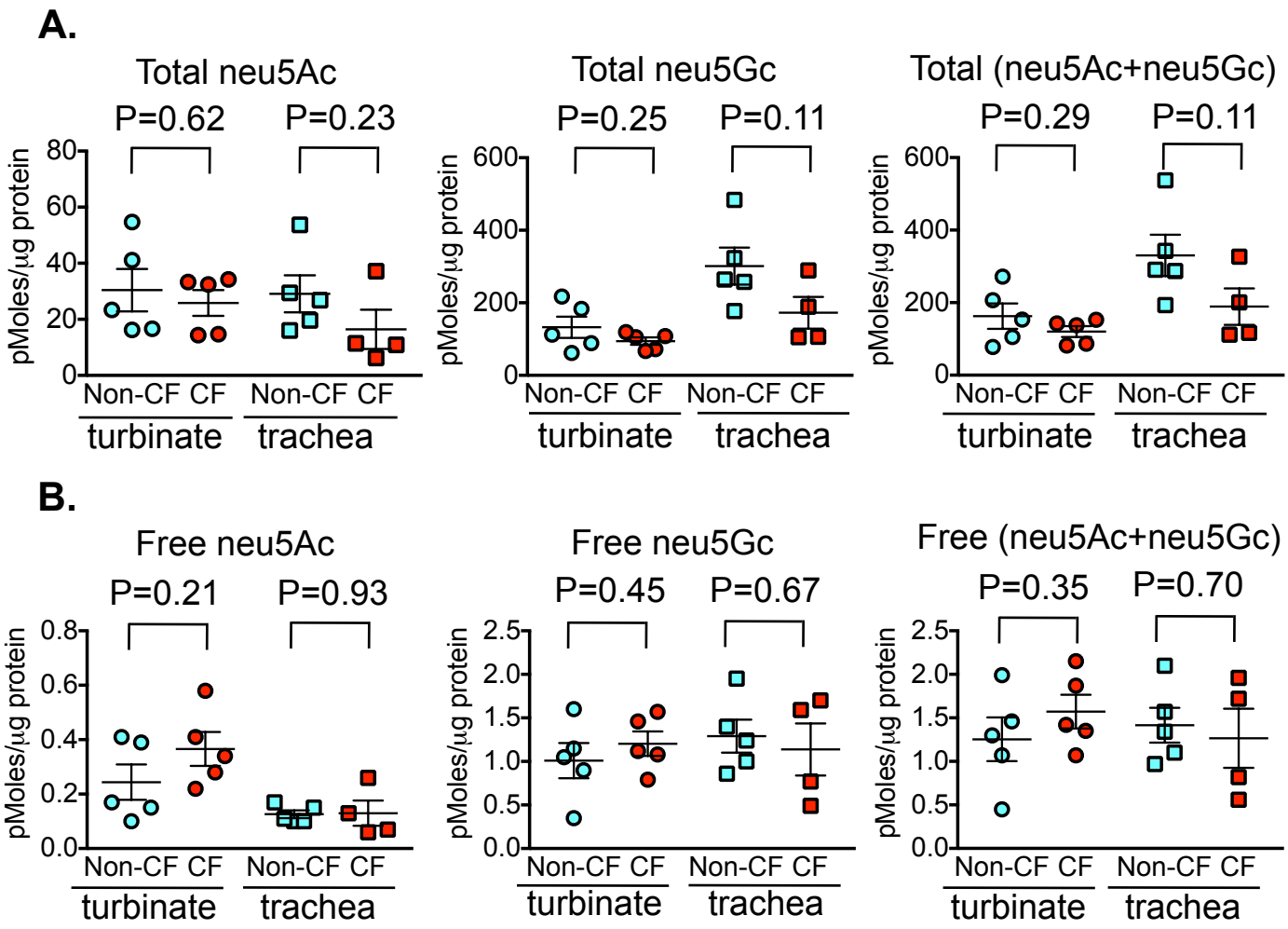

Figure S1. Total and free sialic acid in ASL from cultured nasal and tracheal epithelia.

Total **(A)** and free **(B)** sialic acids (neu5Ac and neu5Gc) in ASL from cultured airway epithelia were determined by HPAE-PAD. N=4-5 per genotype, each from a different pig. Bars indicate means±SEM. P values were by unpaired Student's t-test.

Fig. S1

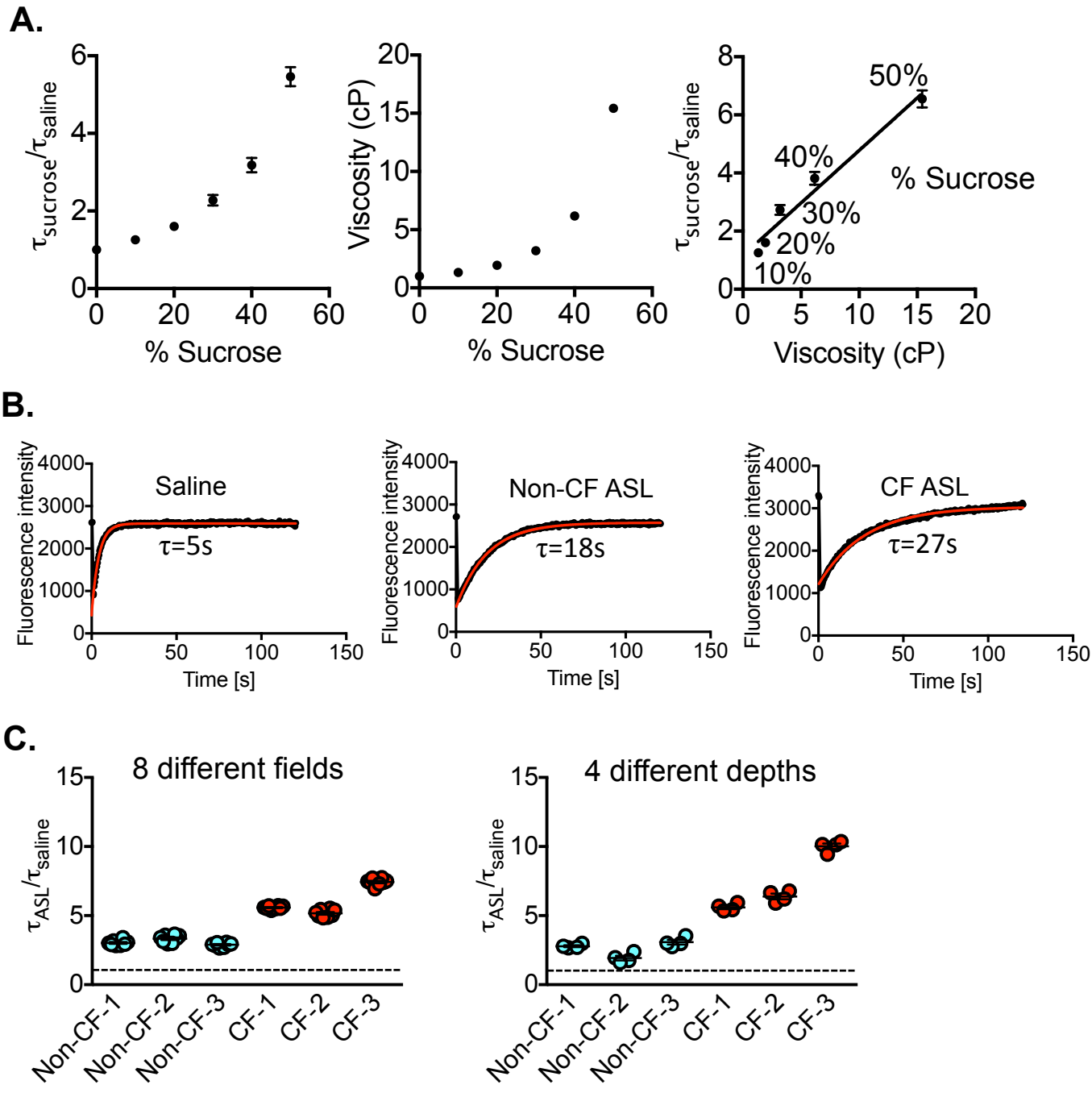

Figure S2. Fluorescence recovery after photobleaching (FRAP) to assess ASL viscosity. (A) $\tau_{sucrose}/\tau_{saline}$ (left) by FRAP and viscosity (middle) determined by cone and plate viscometer (DR Lide, CRC Handbook of Chemistry and Physics. CRC Press. 2000.) for solutions containing increasing sucrose concentrations. Comparison of $\tau_{sucrose}/\tau_{saline}$ and viscosity (right). N=4 per condition; R^2=0.94, *P<0.0001. **(B)** Representative FRAP traces (black) with the fitted line (red) for saline and for ASL of non-CF and CF cultured airway epithelia. **(C)** τ_{ASL}/τ_{saline} measured in 8 different microscopic fields (left panel) and 4 different depths (right panel) in CF and non-CF cultured airway epithelia (N=3 epithelia per group, each from a different pig). Bars indicate means±SEM.

Fig. S2

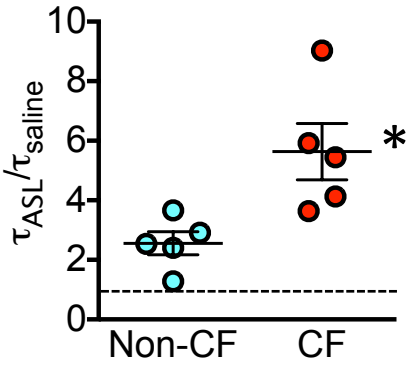

Figure S3. ASL viscosity in differentiated primary airway epithelial cultures apically washed 1 week before measurement. N=5 per genotype, each from a different pig. Bars indicate means±SEM. Asterisk indicates $P<0.05$ by unpaired Student's t-test.

Fig. S3

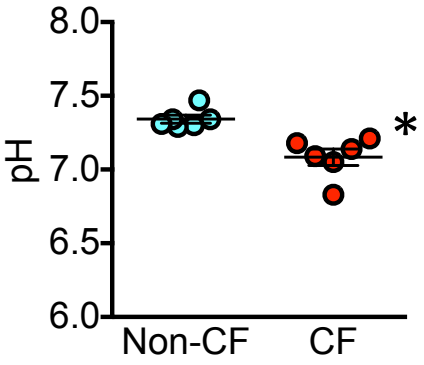

Figure S4. ASL pH in differentiated primary airway epithelia cultured from non-CF and CF humans. N=6 cultures per genotype, each from a different human donor. Bars indicate means±SEM. Asterisk indicates $P<0.05$ by unpaired Student's t-test. See also Fig. 2C.

Fig. S4

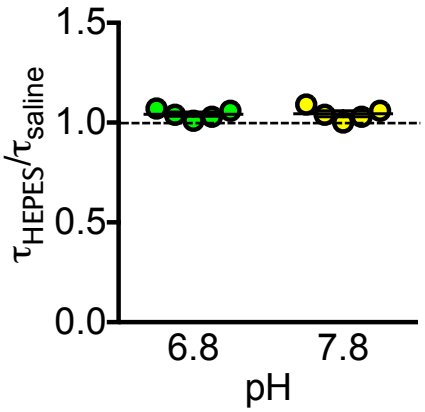

Figure S5. Viscosity of HEPES buffer at different pH. $\tau_{HEPES}/\tau_{saline}$ of 20 mM HEPES buffer in saline at pH 6.8 and 7.8. Bars indicate means±SEM. See also Fig. 5D.

Fig. S5

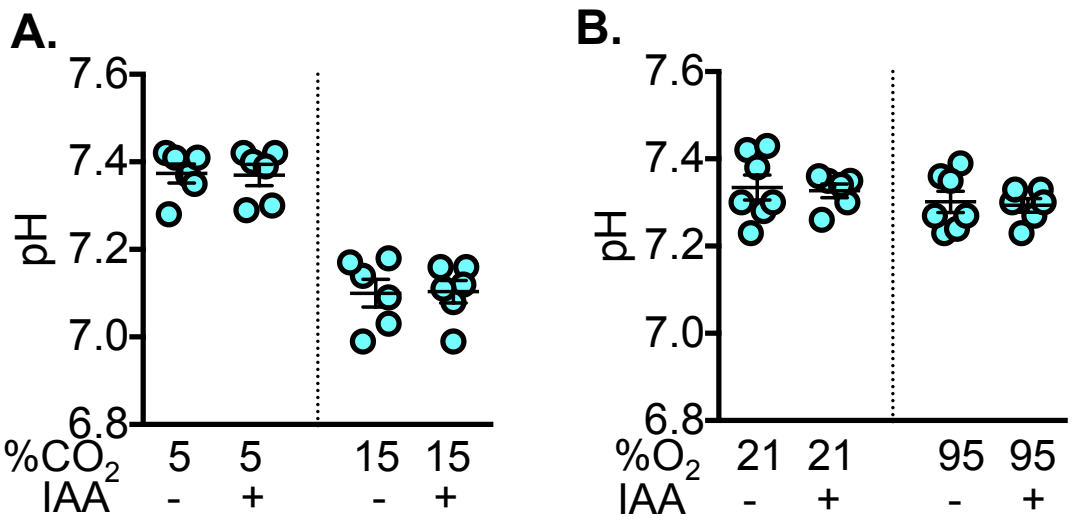

Figure S6. Effect of iodoacetamide (IAA) on ASL pH of cultured non-CF airway epithelia. **(A)** 25 mM iodoacetamide (IAA) was added to non-CF airway epithelia exposed to 5% or 15% CO_2. N=6 epithelia per condition, each from a different pig. See also Fig. 6C. **(B)** 25 mM IAA was added to non-CF airway epithelia exposed to 21% or 95% O_2. N=6-7 epithelia per condition, each from a different pig. See also Fig. 6D. Bars indicate means±SEM.

Fig. S6

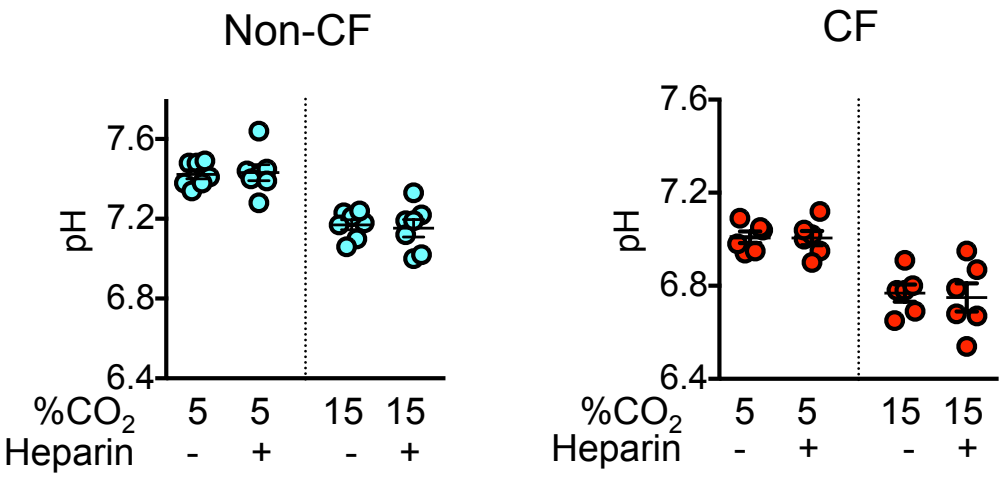

Figure S7. Effect of heparin on ASL pH of non-CF and CF cultured airway epithelia.

Heparin (1 mg/ml in 3 μl of PBS) was added to ASL of non-CF or CF cultured airway epithelia exposed to 5 or 15% CO_2. N=6 or 7 per condition, each from a different pig. See also Fig. 7A. Bars indicate means±SEM.

Fig. S7

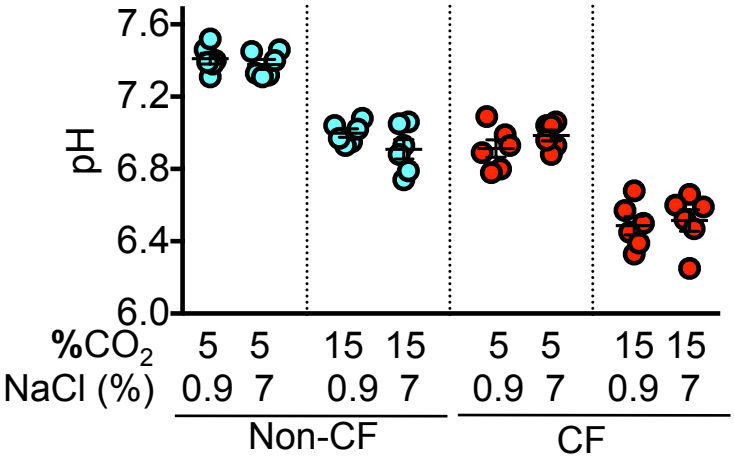

Figure S8. Effect of 0.9% and 7% NaCl on pH of ASL collected from newborn non-CF and CF pigs. 4 μl of 0.9% or 7% NaCl was added to 10 μl ASL collected from non-CF and CF newborn pigs simulated with methacholine. ASL was exposed to 5% or 15% CO_2. N=6 per condition, each from a different pig. See also Fig. 7B. Bars indicate means±SEM.

Fig. S8

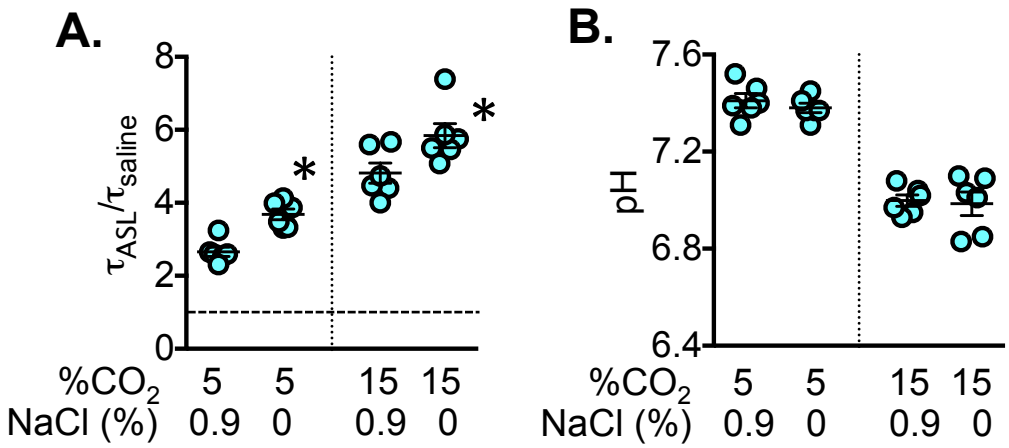

Figure S9. Effect of 0% and 0.9% NaCl on τ_{ASL}/τ_{saline} and pH of ASL from non-CF newborn pigs. 4 μl of 0.9% NaCl or water was added to 10 μl ASL collected from newborn non-CF pigs stimulated with methacholine. ASL was exposed to 5% or 15% CO_2. N=6 per condition, each from a different pig. Bars indicate means±SEM. Asterisk indicates $P<0.05$ by unpaired Student's t-test.

Fig. S9

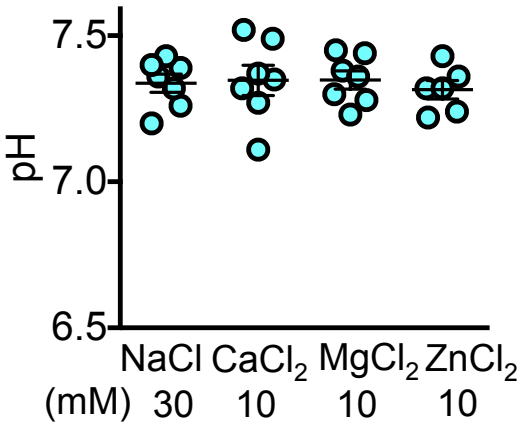

Figure S10. Effect of divalent cations on ASL pH. 30 mM NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$ or 10 mM $ZnCl_2$ in saline (20 mM HEPES, pH 7.35) was added to ASL removed from newborn non-CF pigs. N=6-7 per condition, each from a different pig. See also Fig. 8B. Bars indicate means ± SEM.

Fig. S10

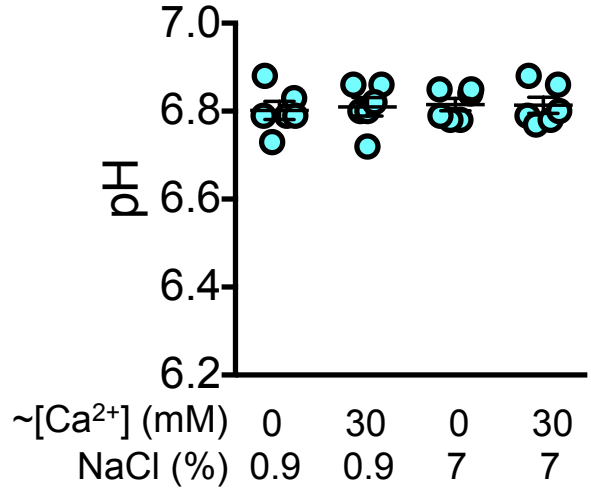

Figure S11. Effect of 0.9% NaCl, 7% NaCl, EGTA and $CaCl_2$ on ASL pH. ASL was collected from newborn non-CF piglets and studied after addition of 20 mM EGTA (0 mM Ca^{2+}), or 100 mM $CaCl_2$ (30 mM Ca^{2+} calculated as described for panel A in Fig. 8). Additions were in 4 μl of 0.9% or 7% NaCl containing 20 mM HEPES at pH 6.8. N=6 per condition, each from a different pig. See also Fig. 8C. Bars indicate means±SEM.