

Supplementary Figures and Tables to:

Basal Cell Expansion Conditions Influence Differentiation and Phenotypic Outcomes in Primary Nasal Epithelia Grown at Air-Liquid Interface

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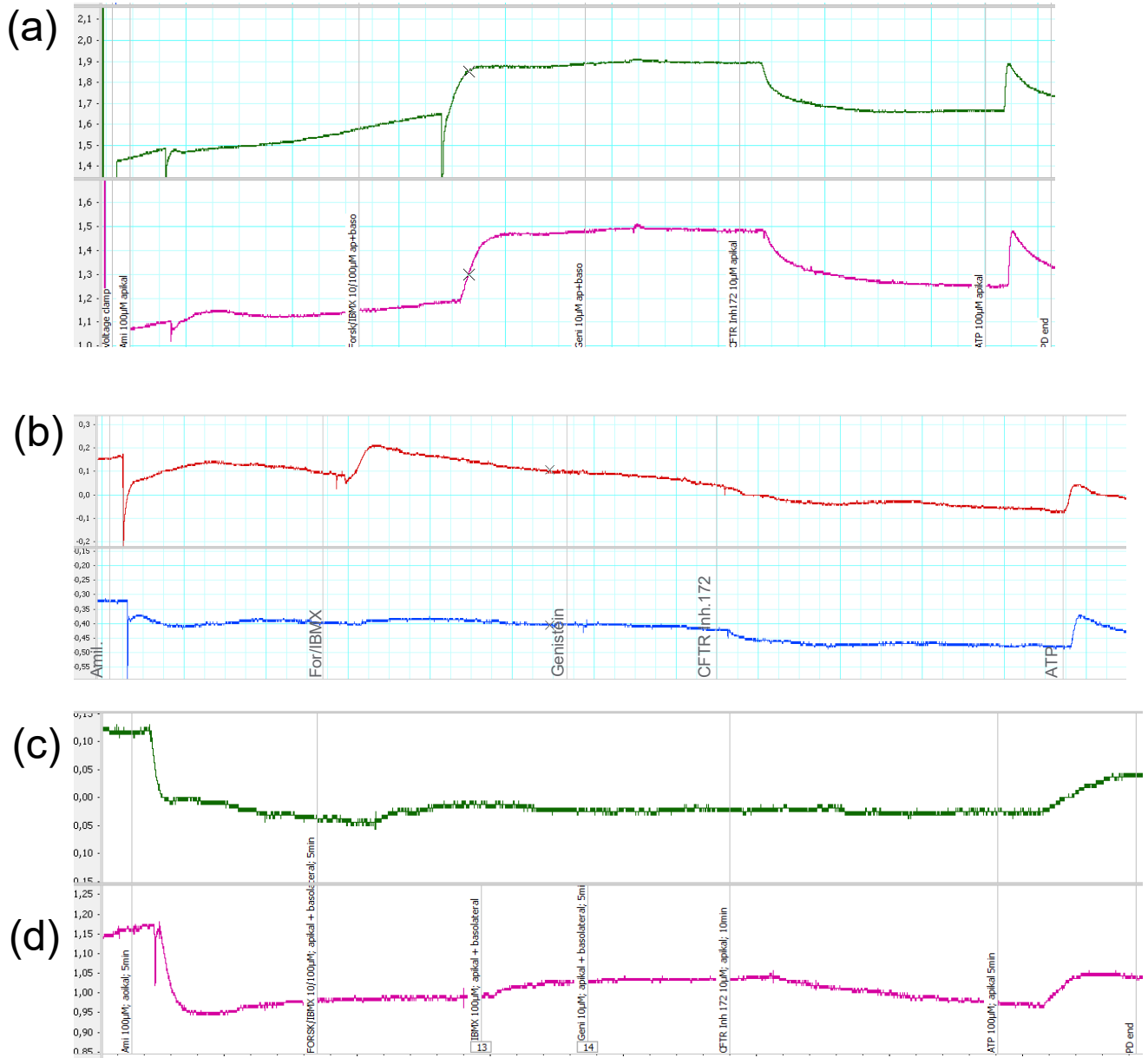
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Supplemental Table S1: Comparison of basal cell expansion media

Component Category	Modified Keratinocyte Medium (In-house)	PneumaCult™-Ex Plus + ROCK/SMAD Inhibitors (ECFS SOP)
Basal Medium	Defined Keratinocyte-SFM – chemically defined, serum-free, BPE-free basal formulation	PneumaCult™-Ex Plus Basal Medium (STEMCELL) – chemically defined, serum-free, BPE-free basal formulation
Growth Factors & Supplements	EGF (0.2 ng/mL; mitogenic signaling), BPE (25 µg/mL; broad growth factor/hormone mix), Isoproterenol (1 µM; β-adrenergic support)	Human Insulin (metabolic/growth support), Transferin (iron homeostasis), Hydrocortisone (0.2 µM; differentiation/barrier modulation), NAC (antioxidant protection)
Pathway Modulators	None	Y-27632 (10 µM), DMH-1 (1 µM), A-83-01 (1 µM)
Primary Experimental Variable	Basal expansion medium without pathway inhibitors	Identical basal expansion medium + ROCK/SMAD pathway inhibitors

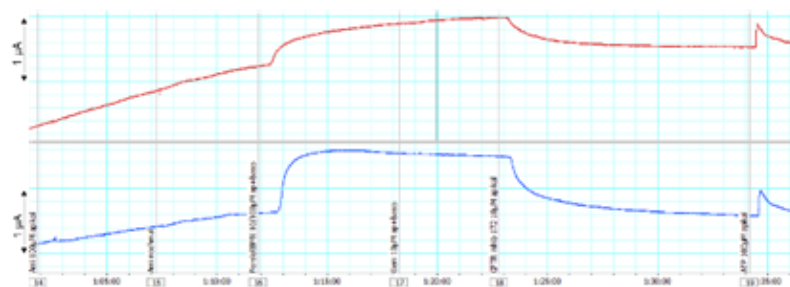


Supplemental Figure S1. Recordings using different chloride gradients for Ussing chamber measurements. All recordings are from one batch of expansion cultures, derived from the same biomaterial expanded from lung transplant airways, which is routinely used at our site to control the Ussing chamber recordings prior to measuring patient's samples for diagnostic purposes. (a) two recordings using a steep gradient as described by Noel et al [4] of 0 mM chloride (apical) / 145 mM chloride (basolateral); (b) both sides with equal ion concentration using Ringer solution (c) both sides with equal chloride concentration of 115 mM chloride (d) low gradient with 15 mM chloride (apical) / 115 mM chloride (basolateral). In (a) and (d), chloride is wholly (apical, in (a)) or partially (apical in (d)) compensated for with gluconate.

seeding directly on inserts
(P0)

2

expansion
WITHOUT
ROCK/SMAD-
inhibitors

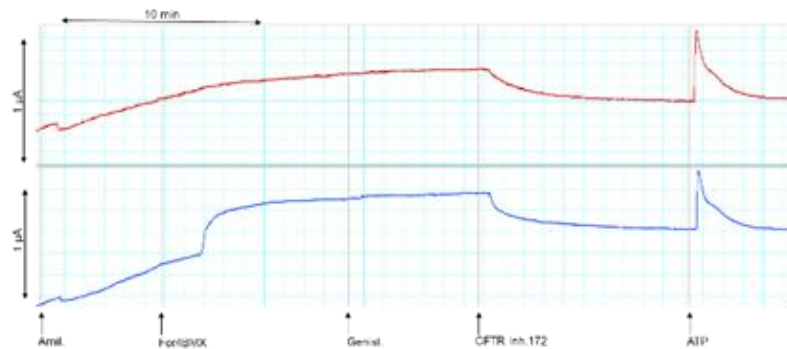


Supplemental Figure S2. Large-scale presentation of Tracings in Figure 5 (Ion transport measurements in a micro Ussing-chamber), part 1 of 4. See legend to Figure 5 for details.

seeding directly on inserts
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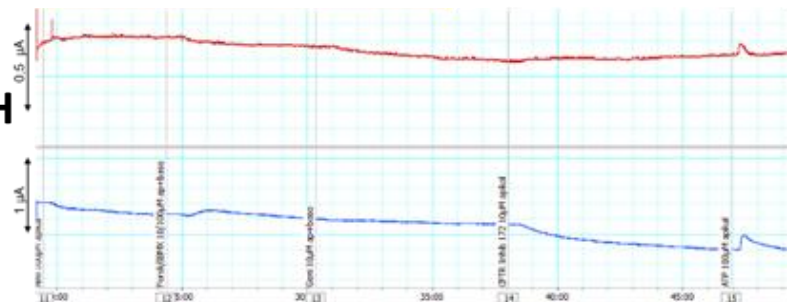
8

expansion
WITHOUT
ROCK/SMAD-
inhibitors



8

expansion **WITH**
ROCK/SMAD-
inhibitors

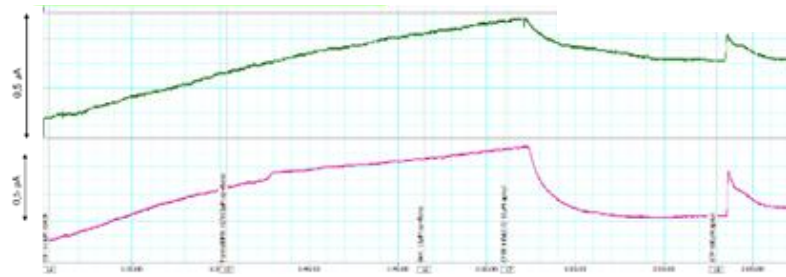


Supplemental Figure S3. Large-scale presentation of Tracings in Figure 5 (Ion transport measurements in a micro Ussing-cham-ber), part 2 of 4. See legend to Figure 5 for details.

seeding directly on inserts
(P0)

6

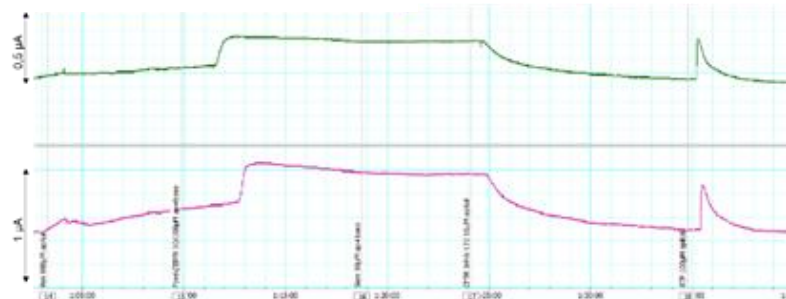
expansion
WITHOUT
ROCK/SMAD-
inhibitors



6



expansion **WITH**
ROCK/SMAD-
inhibitors

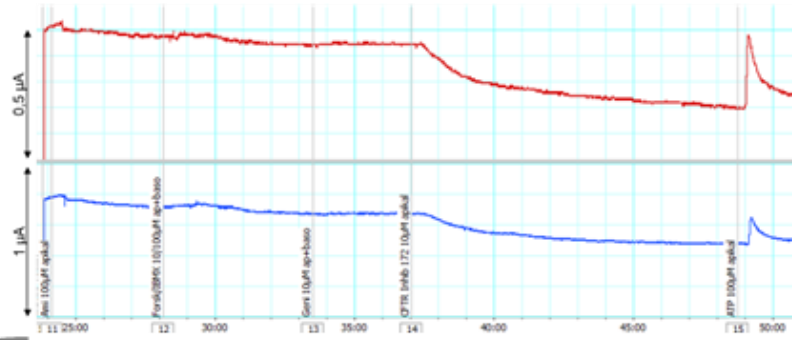


Supplemental Figure S4. Large-scale presentation of Tracings in Figure 5 (Ion transport measurements in a micro Ussing-cham-ber), part 3 of 4. See legend to Figure 5 for details.

passaging prior to seeding
on inserts (P1)

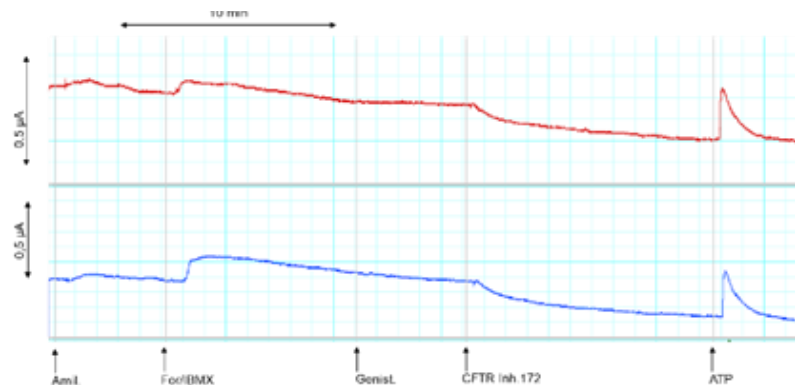
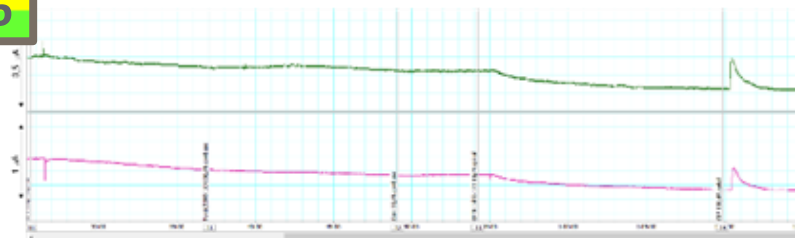
1

expansion
WITHOUT
ROCK/SMAD-
inhibitors



6

expansion **WITH**
ROCK/SMAD-
inhibitors



Supplemental Figure S5. Large-scale presentation of Tracings in Figure 5 (Ion transport measurements in a micro Ussing-chamber), part 4 of 4. See legend to Figure 5 for details.