

PneumaCult™-Ex Plus, a Novel Defined and Feeder-Free Medium, Supports the Improved Expansion of Primary Human Airway Epithelial Cells and Maintenance of their Mucociliary Differentiation Potential in Later Passages

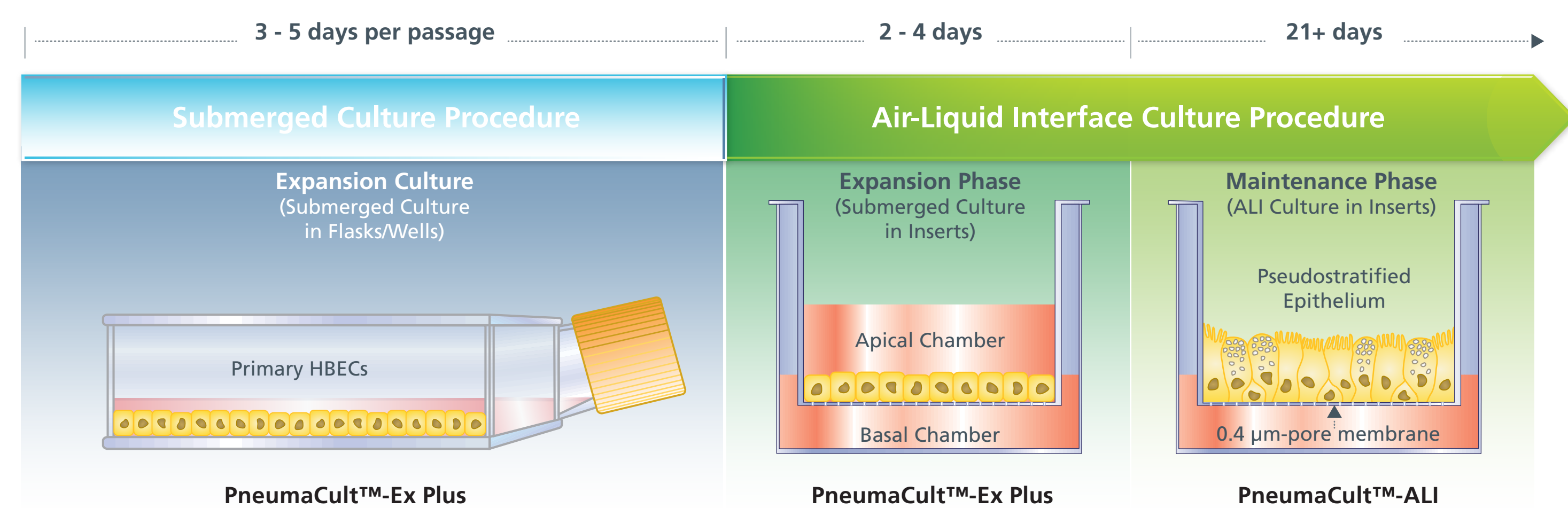
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Introduction

Traditional feeder-free and Bovine Pituitary Extract (BPE)-containing media formulations for the expansion of primary human bronchial epithelial cells (HBECS) typically support the maintenance of their mucociliary differentiation potential for a limited number of passages in vitro. A novel culture system comprising an inactivated mouse embryonic fibroblast feeder layer and a specialized medium has been recently reported to improve the expansion of HBECS while maintaining their differentiation potential, even after extended passaging^{1,2}. However this feeder-dependent method is cumbersome and undefined, thus limiting its utility. We have developed a novel feeder- and BPE-free culture medium, PneumaCult™-Ex Plus, that promotes extended passaging of HBECS without the loss of their differentiation potential at later passages. PneumaCult™-Ex Plus allows for the rapid expansion of HBECS, while maintaining their ability to form a pseudostratified mucociliary epithelium at air-liquid interface (ALI) for more passages, compared to other commercial HBEC expansion media.

Materials and Methods

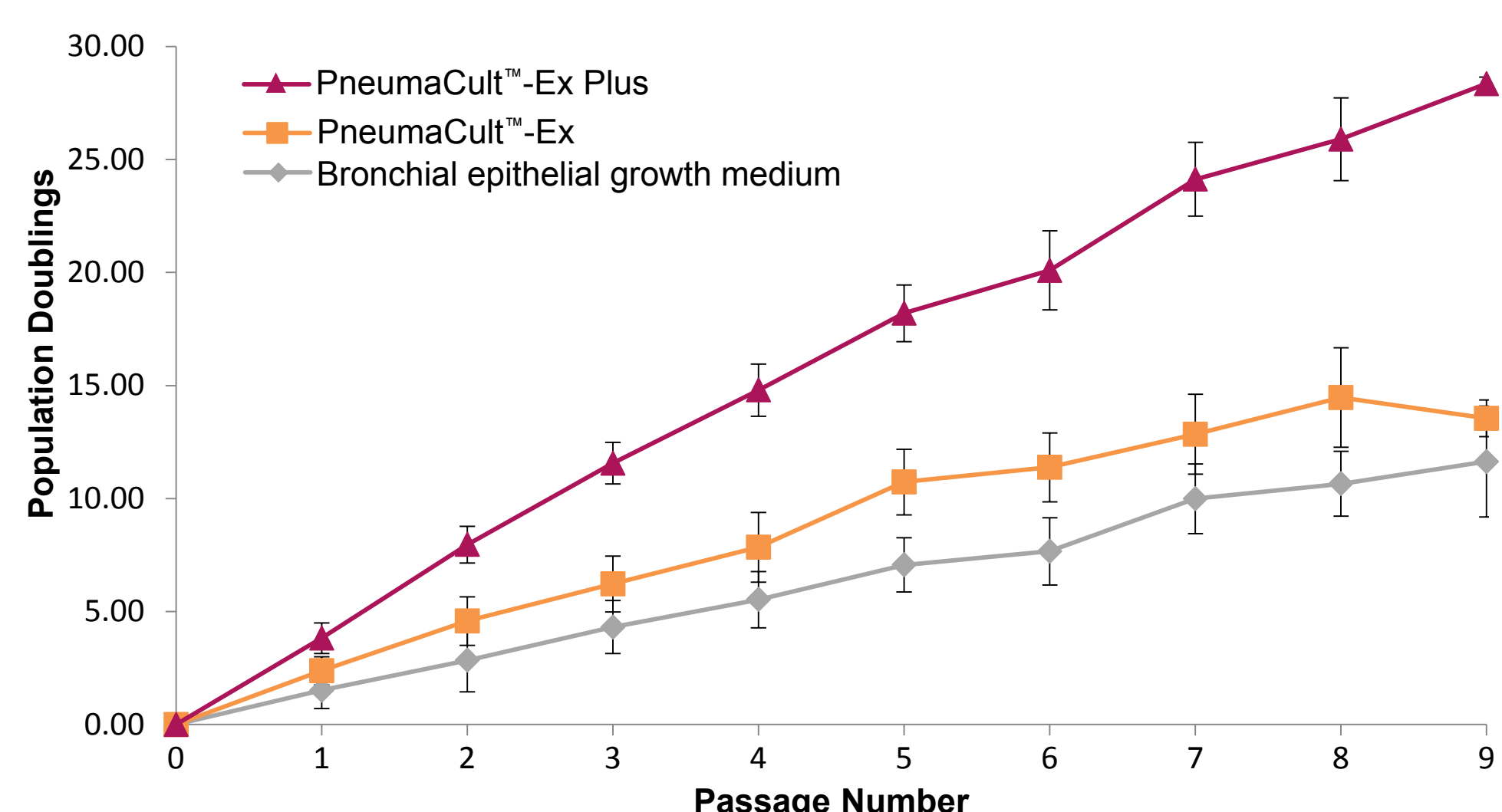
FIGURE 1: PneumaCult™ culture system



Commercially available primary normal human airway epithelial cells such as Lonza's HBECS [Passage (P)1; Catalog #CC-2540s] were thawed and seeded directly into T-25 cm² culture flasks containing either PneumaCult™-Ex Plus, PneumaCult™-Ex or commercial bronchial epithelial growth medium at a density of 3.5 x 10³ cells/cm². Culture media were fully replenished every other day and cultures were passaged once cells reached approximately 80% confluence. At each passage, the cells were enzymatically dissociated using Trypsin-EDTA (0.05%) and then re-plated at a density of 5 x 10³ cells/cm² in the different medium. Fold expansion was measured over 8 passages and the differentiation potential was assessed at each passage.

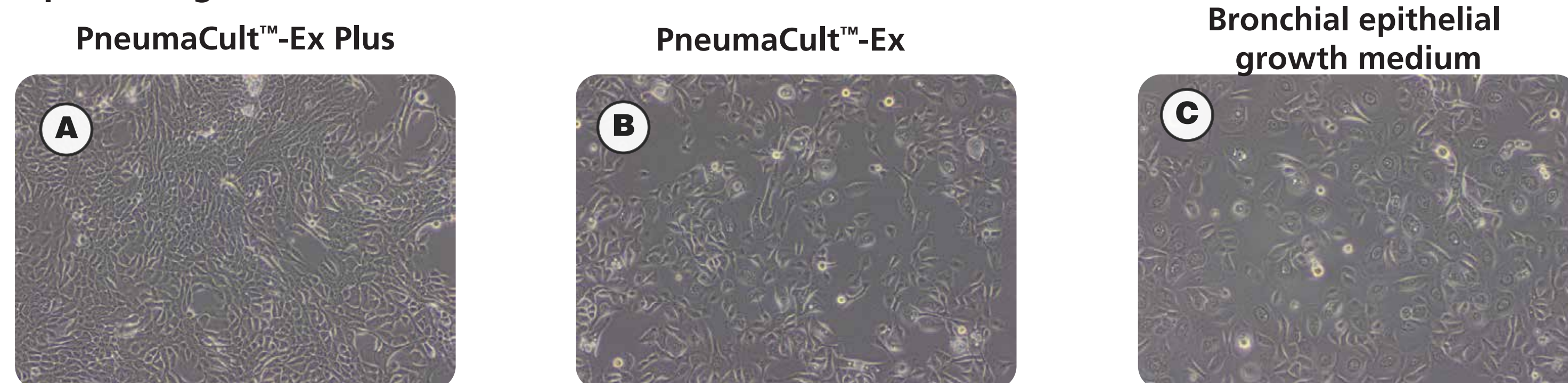
Results

FIGURE 2: HBECS cultured in PneumaCult™-Ex Plus showed more rapid expansion compared to those cultured in PneumaCult™-Ex and commercial bronchial epithelial growth medium



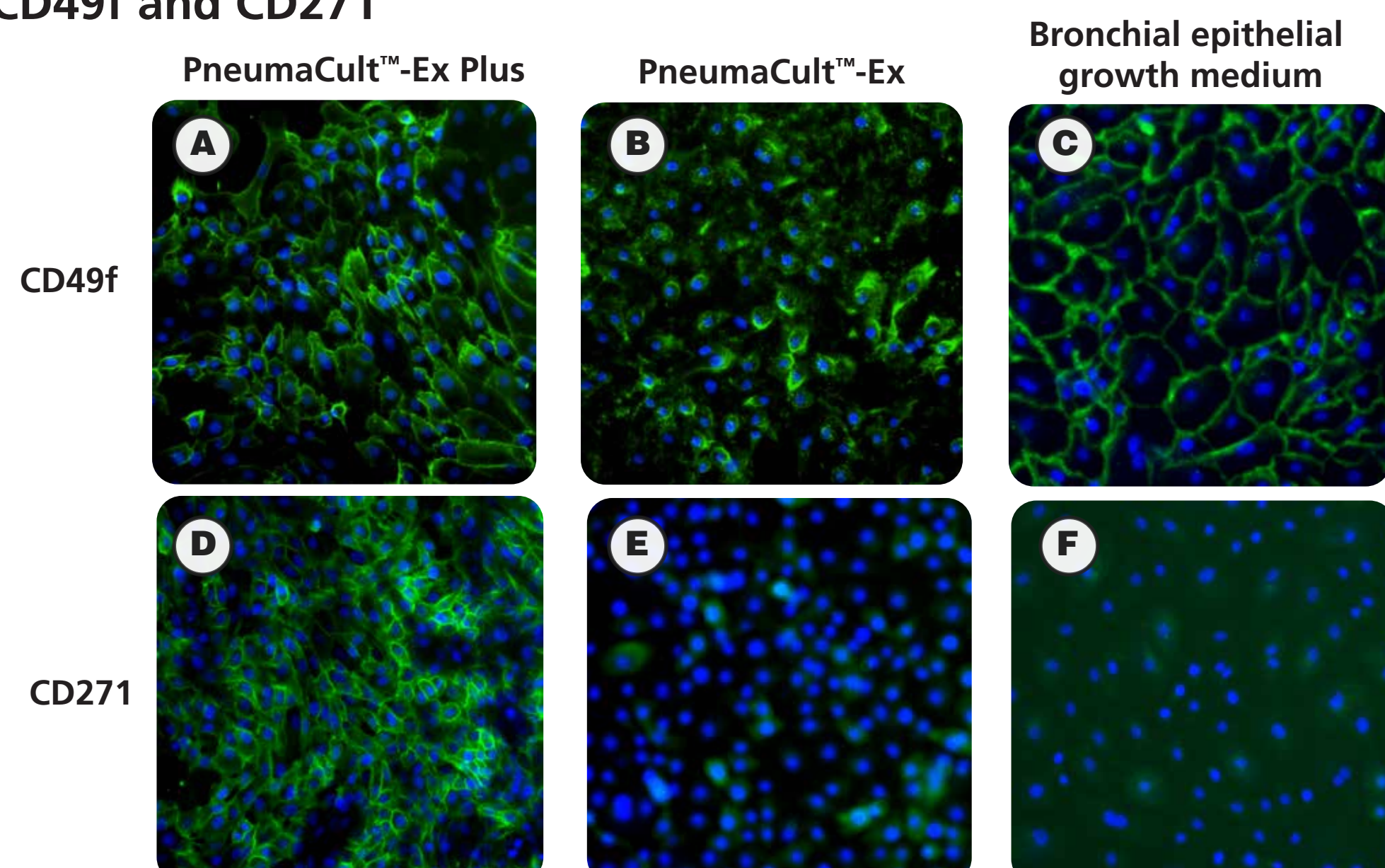
Commercially available, cryopreserved, passage 1 (P1) HBECS were seeded into PneumaCult™-Ex Plus, PneumaCult™-Ex or commercial bronchial epithelial growth medium. Cells cultured in PneumaCult™-Ex Plus have a significantly higher proliferation rate over 9 passages compared to those maintained in either PneumaCult™-Ex or commercial bronchial epithelial growth medium (n=6).

FIGURE 3: Representative morphology of HBECS cultured in PneumaCult™-Ex Plus, PneumaCult™-Ex, or Bronchial epithelial growth medium



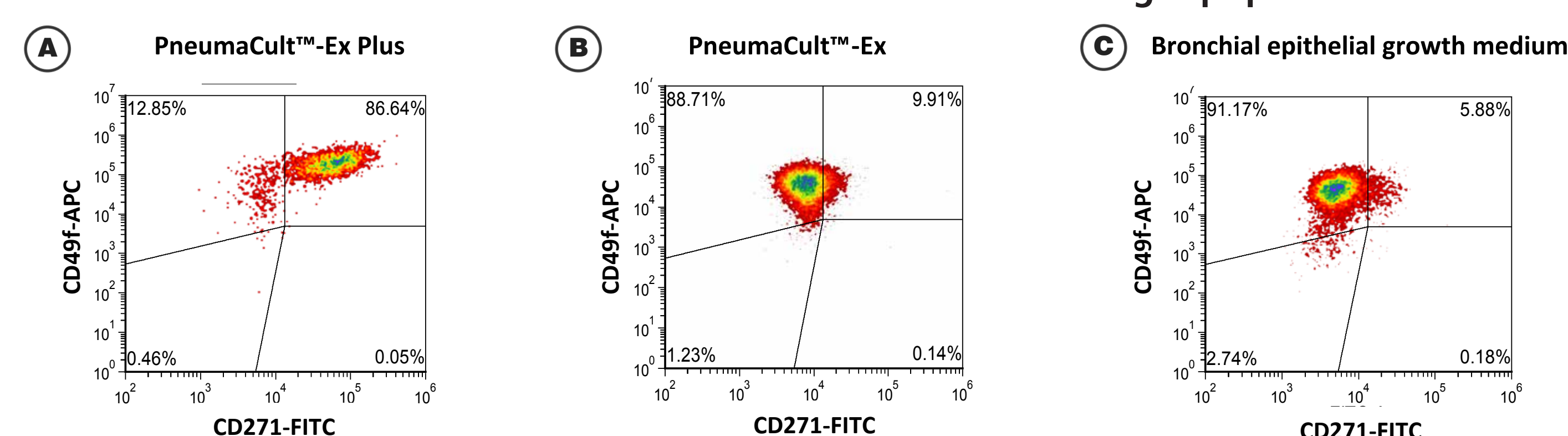
Representative live culture images for P4 HBECS cultured in PneumaCult™-Ex plus or control media (PneumaCult™-Ex or commercial bronchial epithelial growth medium). Compared to the cells cultured in PneumaCult™-Ex (B) and commercial bronchial epithelial growth medium (C), HBECS cultured in PneumaCult™-Ex Plus (A) are smaller and more tightly packed. All images were taken using 10X objective.

FIGURE 4: HBECS cultured in PneumaCult™-Ex Plus maintain widespread expression of the basal cell markers CD49f and CD271



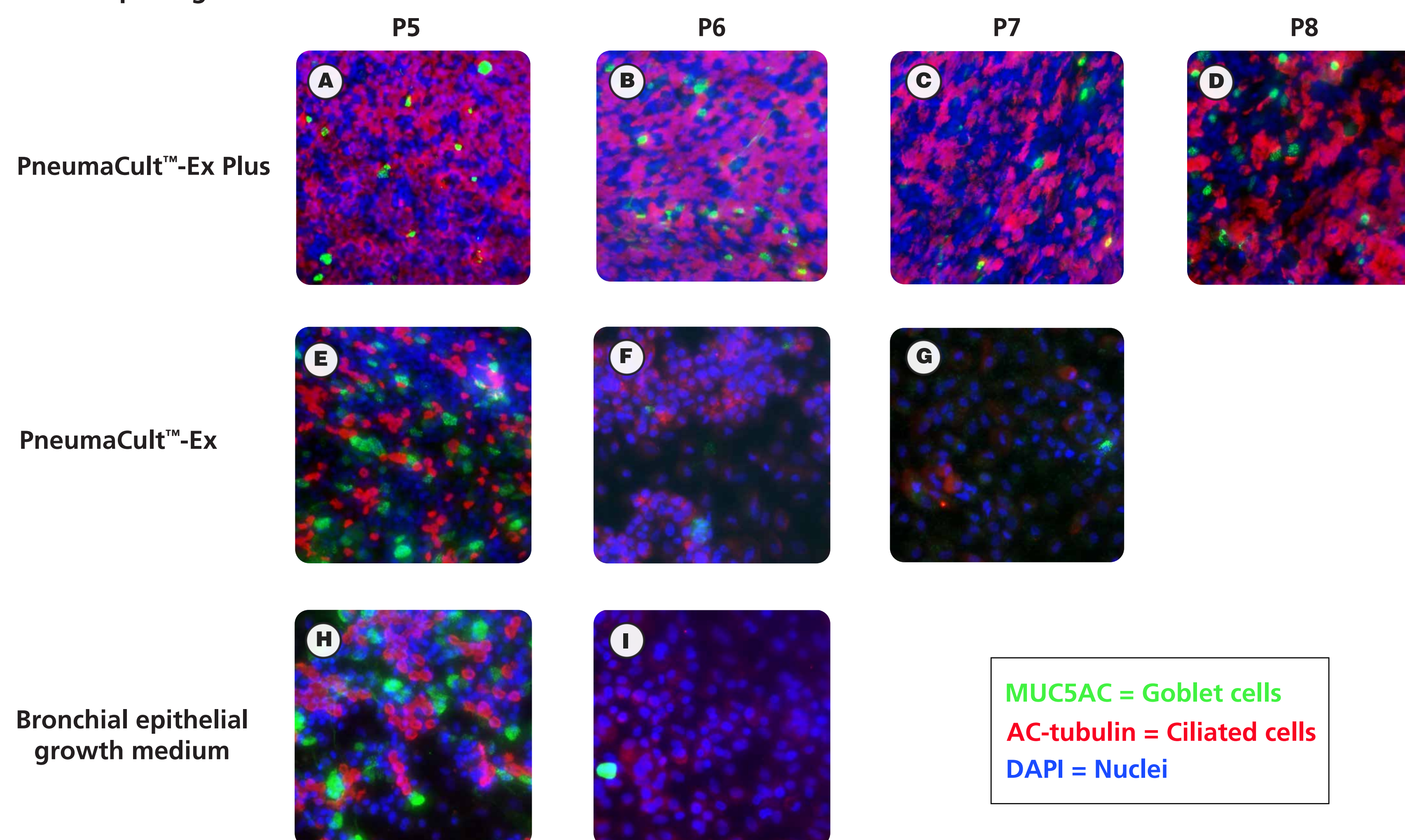
Immunocytochemistry detection of basal cell markers CD49f (A, B, and C) and CD271 (D, E, and F) for P4 HBECS cultured in PneumaCult™-Ex Plus (A and D), PneumaCult™-Ex (B and E), and commercial bronchial epithelial growth medium (C and F). All images were taken using 10X objective. Nuclei are counterstained with DAPI.

FIGURE 5: HBECS cultured in PneumaCult™-Ex Plus maintain a larger population of CD271⁺CD49f⁺ basal cells



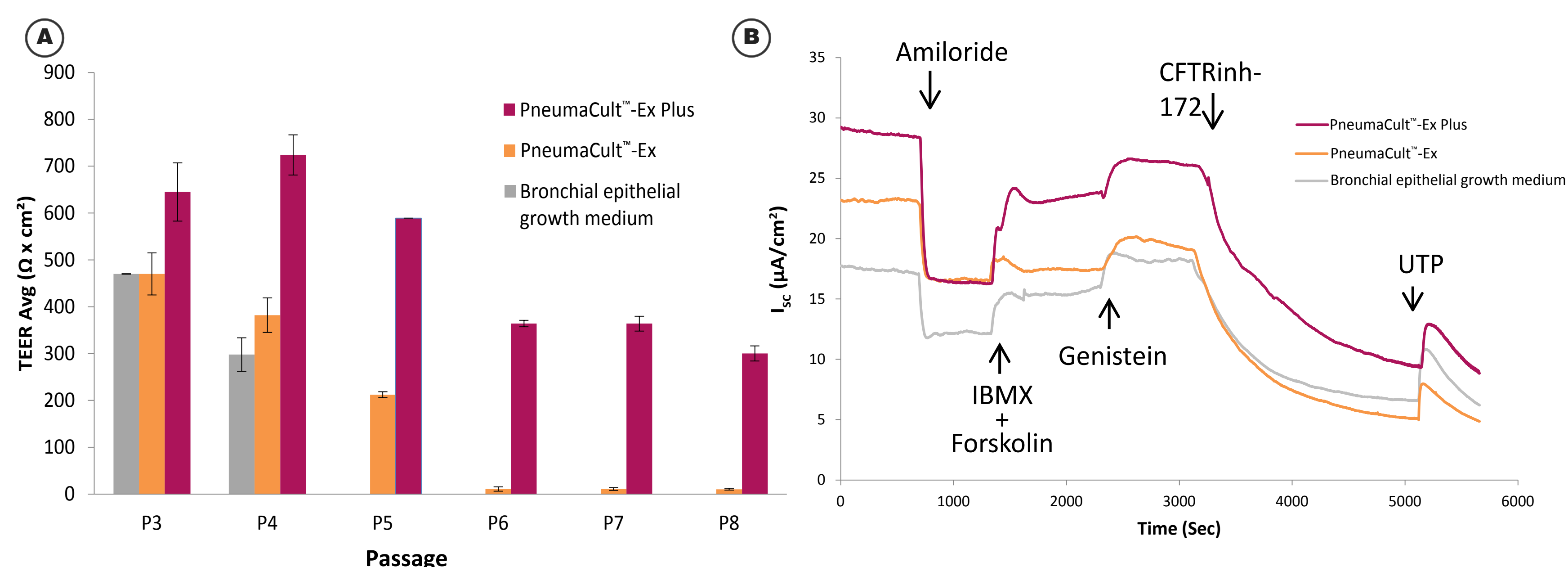
P4 HBECS cultured in PneumaCult™-Ex Plus (A), PneumaCult™-Ex (B), and commercial bronchial epithelial growth medium (C) were characterized by flow cytometry for expression of the basal cell markers CD49f and CD271. HBECS cultured in PneumaCult™-Ex Plus (A) have a higher percentage of cells co-expressing CD49f and CD271 compared to those cultured in PneumaCult™-Ex (B) and commercial bronchial epithelial growth medium (C).

FIGURE 6: HBECS cultured in PneumaCult™-Ex Plus differentiate into a pseudostratified mucociliary epithelium at later passages in PneumaCult™-ALI



P4 HBECS were seeded and passaged in either PneumaCult™-Ex Plus, PneumaCult™-Ex, or commercial bronchial epithelial growth medium, followed by ALI differentiation at each passage (P5 - 8) with PneumaCult™-ALI Medium. The ALI cultures at 28 days post air-lift were fixed and stained with antibodies for cilia marker acetylated-tubulin (red) and the goblet cell marker MUC5AC (green). The nuclei are counterstained with DAPI (blue). All images were taken using 20X objective.

FIGURE 7: Electrophysiological characterization of differentiated HBECS previously expanded in PneumaCult™-Ex Plus, PneumaCult™-Ex, and commercial bronchial epithelial growth medium



(A) Transepithelial electrical resistance (TEER) for ALI cultures at 28 days post air-lift using HBECS expanded in either PneumaCult™-Ex Plus, PneumaCult™-Ex, or commercial bronchial epithelial growth medium. (B) Representative characterization of the ion channel activities for the ALI cultures at 28 days post air-lift using HBECS expanded in PneumaCult™-Ex Plus, PneumaCult™-Ex, or commercial bronchial epithelial growth medium. Amiloride: Epithelial Sodium Channel (ENaC) inhibitor. IBMX and Forskolin: Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) activators. Genistein: CFTR potentiator. CFTRinh-172: CFTR inhibitor. UTP: Calcium-activated Chloride channels (CaCCs) activator. I_{sc}: Short Circuit Current. All ALI differentiation cultures were performed using PneumaCult™-ALI Medium.

Conclusions

- PneumaCult™-Ex Plus is a serum- and BPE- free medium that supports higher expansion of HBECS compared to a commercial expansion media
- HBECS expanded in PneumaCult™-Ex Plus maintain better mucociliary differentiation potentials
- PneumaCult™-Ex, together with PneumaCult™-ALI, creates an optimized BPE-free culture system for expanding and differentiating HBECS into a pseudostratified mucociliary epithelium, resembling the human airway both morphologically and electrophysiologically

References

1. Liu X, et al. (2012) Am J Pathol 180(2): 599-607
2. Supryniewicz F, et al. (2012) PNAS (109): 20035-20040