**Supplemental Table I. Summary of Immortalized Human Airway Epithelial Cell Lines[1]**

| Name | Immortalization method | Donor material | Differentiation conditions | Differentiation capacity | References |
|---|---|---|---|---|---|
| BEAS-2B | Hybrid adeno-SV40 virus | Bronchial epithelium from explants of autopsy specimens from noncancerous individuals | Submerged culture | Squamous differentiation | 1 |
| | | | ALI[2] culture | No ciliated or secretory cells | 2 |
| HTEo- | Origin defective SV40 genome | Enzymatically isolated tracheal cells or outgrowths of tracheal tissue explants | Not reported | Not reported | 3 |
| 16HBE14o- | Origin defective SV40 genome | Bronchial surface epithelial cells from a 1yr-old male heart-lung transplant patient | ALI culture with vitrogen coated support and serum containing media | Ciliated cells | 4 |
| hTBE | SV40 early region and hTERT | Tracheal and bronchial epithelium from lung transplants | Not reported | Not reported | 5 |
| SA (SV40 ER + hTERT) | SV40 early region and hTERT | "Small airway epithelial cells" expressing cytokeratin 19 from commercial source[3] | Subcutaneous injection of nude mouse | Histology similar to normal airway epithelium | 5 |
| NuLi-1-2 | HPV-16 E6/E7 and hTERT | Bronchial epithelium enzymatically isolated from healthy donor lungs | ALI culture with collagen coated support and ultroser G containing media | NuLi-2 differentiated into goblet and ciliated cells | 6 |
| HBEC1-4 | CDK4 and hTERT | Bronchial epithelium from areas of the lung histologically not involved with lung cancer | ALI culture with IMR90 fibroblast-contracted collagen gel | HBEC3 cells differentiated into goblet and ciliated cells | 7-9 |
| | | | 3D matrigel co-culture with IMR90 fibroblasts | HBEC3 cells produce spheroid cyst-like structures | 10 |
| | | | 2D submerged monolayer culture for 2 days in differentiation media | HBEC3 cells simultaneously express markers for basal, Clara and type II pneumocytes | 10 |

**Supplemental Table I. Summary of Immortalized Human Airway Epithelial Cell Lines (cont. page 2)**

| Name | Immortalization method | Donor material | Differentiation conditions | Differentiation capacity | References |
|---|---|---|---|---|---|
| AE-hTERT (F and M) | hTERT | Passage 2 - small airway epithelial cells from one female and one male nonsmoker from commercial source[3] | Not reported | Not reported | 11 |
| VA10 | HPV-16 E6/E7 | Primary bronchial epithelial cells | ALI culture with collagen coated support and ultroser G containing media | Ciliated cells | 12,13 |
| | | | 3D matrigel co-culture model with HUVECs[4] | Produce 3D bronchioalveolar like structures | 14 |
| UNCN1T-3T | Bmi-1 and hTERT | Human bronchial epithelial cells cultured from lung tissue obtained from male and female donors | ALI culture with collagen coated support | All three cell lines differentiated into secretory and ciliated cells | 15 |

[1] Cell lines generated using only normal tissue as primary donor material.
[2] Air-liquid interface.
[3] Bio-Whittaker/Clonetics (Walkersville, MD).
[4] Human umbilical cord vein endothelial cells.

## Supplemental References


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## Supplemental Figure Legends

**Supplemental Figure 1.** Cell viability analysis of parental and clonal immortalized BCi-NS1 cells over continuous serial passage. For both parental and clonal BCi-NS1.1 cell lines, cell viability was assessed using trypan blue exclusion following trypsinization during early and late passages. **A.** Parental BCi-NS1 cells were assessed at eleven early passages (passage 8-20) and eleven later passages (passage 35-46). **B.** Clonal BCi-NS1.1 cells were assessed at twelve early passages (passage 6-23) and twelve later passages (passage 41-71). For both **A.** and **B.,** data shown is the average ± the standard error. Statistics were calculated by 2-tailed Student's t test.

**Supplemental Figure 2.** Growth rate analysis of immortalized BCi-NS1.1 following extended serial passage. BCi-NS1.1 cells from early (passage 9-12); middle (passage 46-49) and late (passage 71-74) passages were seeded in 12-well plates at a density of 3000 cells/$cm^2$ in growth media in a side by side comparison and harvested every 24 hr to assess the growth rate of the cells. Data shown is the average ± the standard error of n=4 independent experiments. Statistics were calculated by 2-tailed Student's t test.

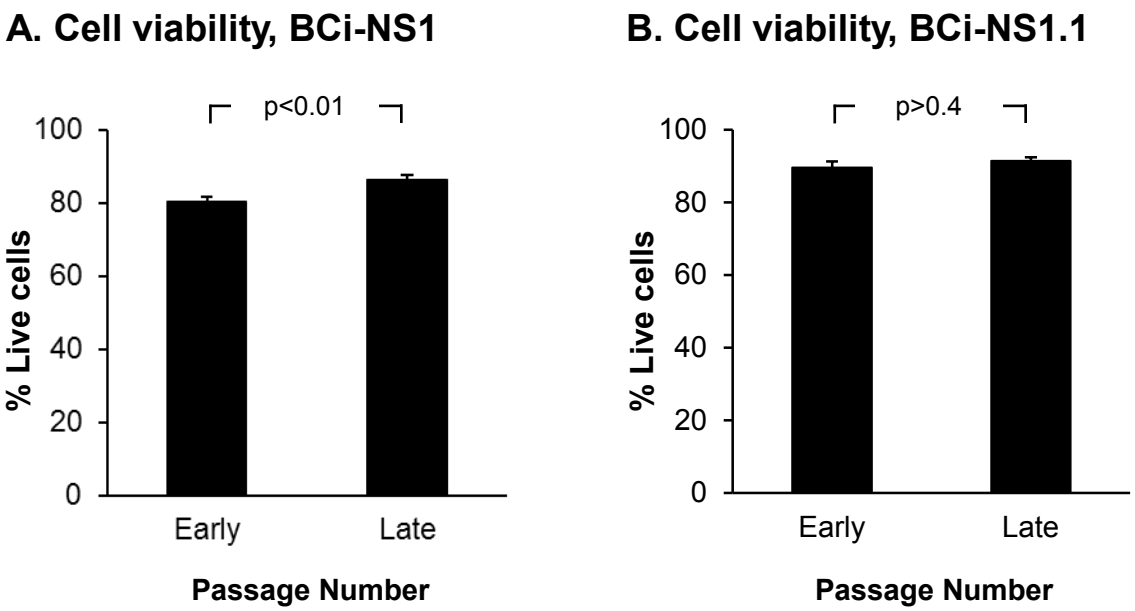
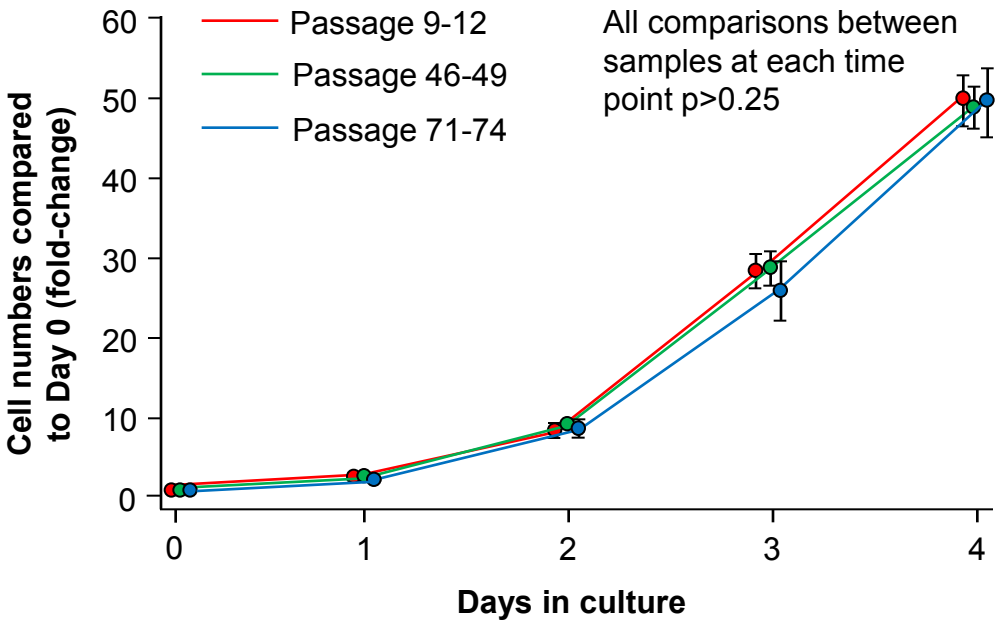

A. Cell viability, BCi-NS1
p<0.01
100
80
60
40
20
0
% Live cells
Early
Late
Passage Number
B. Cell viability, BCi-NS1.1
p>0.4
100
80
60
40
20
0
% Live cells
Early
Late
Passage Number

**Figure S2**


Passage 9-12
Passage 46-49
Passage 71-74
All comparisons between samples at each time point p>0.25
Cell numbers compared to Day 0 (fold-change)
60
50
40
30
20
10
0
0
1
2
3
4
Days in culture