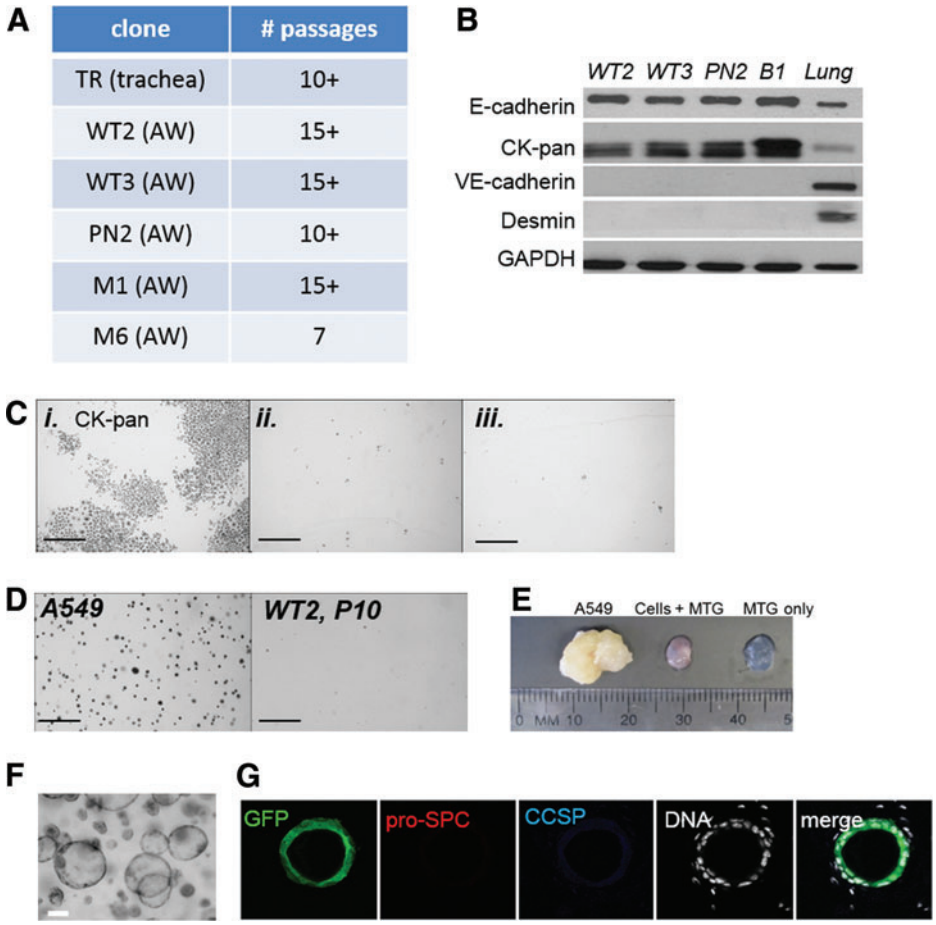

SUPPLEMENTARY FIG. S3. Under low Ca^{2+} conditions, subcultured large colony-derived cells retain epithelial characteristics, growth factor- and substrate-dependency, but lack expression of lung-specific differentiation markers. **(A)** Clones of epithelial cells derived from tracheal (TR) and airway epithelium (AW); the number of subcultures of each clone indicated in the *right column* **(B)** Representative western blot showing expression of selected epithelial and nonepithelial markers in clones subcultured 7–10 times. **(C)** Mitotic shake-off cells from large colonies were cultured for 10 days in low Ca^{2+} media supplemented with: **(Ci)** conditioned media, growth factors, and FBS **(Cii)** growth factors and FBS **(Ciii)** FBS only. Cultures were fixed and stained with anti-pan-cytokeratin antibody. **(D)** Large colony-derived epithelial cells were serially passaged (>seven passages) and plated at a concentration of 10^4 cells/mL in soft agar in complete media for 4 weeks. The A549 cancer cell line (*left*) was used as a positive control. **(E)** Serially passaged epithelial cells were mixed with Matrigel and subcutaneously injected into NSG mice (input: 10^5 cells/200 μL Matrigel per injection). Matrigel plugs recovered 6 weeks after injection of the A549 cancer cell line (*left*) were used as a positive control. **(F)** Representative images of the cysts generated by large colony-derived cells 21 days after plating inside 3D Matrigel. **(G)** Lung $EpCAM^{pos}$ cells were isolated from EGFP transgenic mice and plated in 2D culture to obtain large colonies. GFP-expressing epithelial cells derived from large colonies were mixed with wild-type fibroblasts and cultured in 3D Matrigel. At 21 days postplating, cysts formed by large colony-derived cells were fixed and analyzed for CCSP (*blue*) and pro-SPC (*red*) expression. Scale bars: **C**, 1 mm; **D**, 1 mm; **F**, 100 μm; **G**, 20 μm.