Expanded View Figures

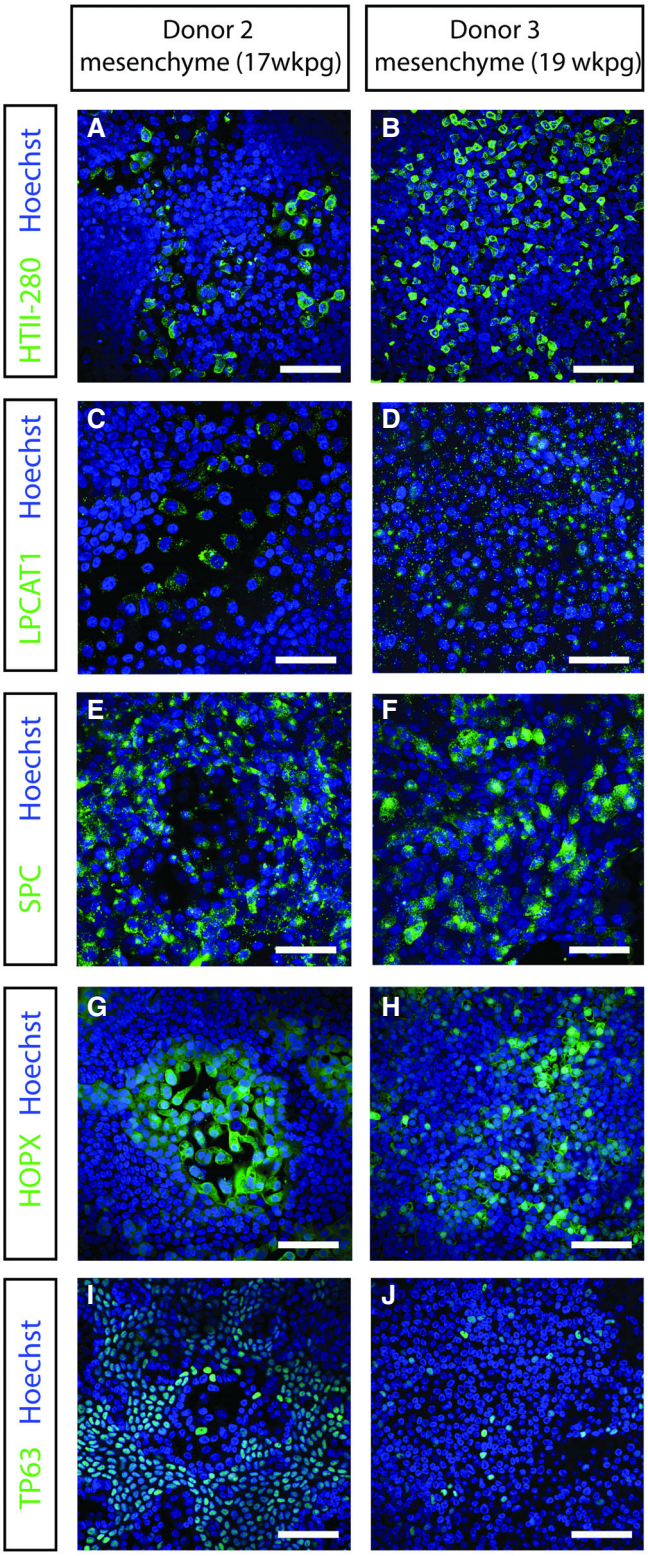

Figure EV1. A comparison of different mesenchymal donors on 2D bronchioalveolar-like differentiation.

A–J Immunofluorescent staining of HTII-280 (A, B), LPCAT1 (C, D), SPC (E, F), HOPX (G, H), and TP63 (I, J) after 2 weeks of differentiation at air–liquid interface in the presence of mesenchyme in the basal compartment of the Transwell. Nuclei are stained with Hoechst (blue). Scale bars represent 50 μm.

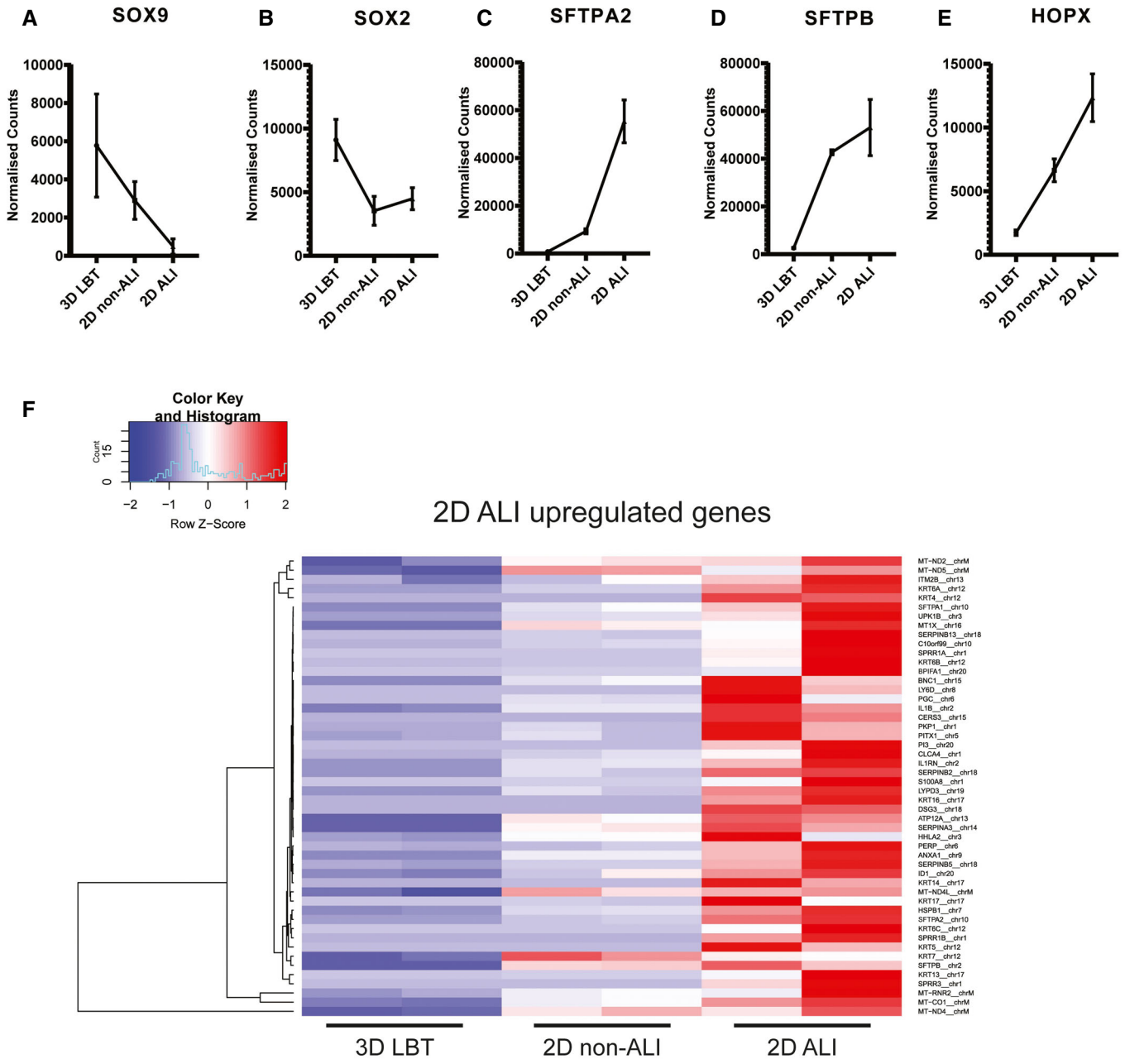

Figure EV2. Transcriptomic analysis of differentiation toward bronchioalveolar-like cells from lung bud tip organoids.

A–E Line graphs indicating normalized read count of 3D lung bud tip organoids (3D LBT), airway organoids on transwell system with apical medium (2D non-ALI), and 2D bronchioalveolar-like air–liquid cultures (2D ALI). $n = 2$ and error bars represent stdev. (A, B) Graphs depicting fetal bud tip progenitor markers SOX9 and SOX2. (C, D) Graphs depicting ATII markers SFTPA2 and SFTPB. (E) Graphs depicting ATI marker HOPX.

F Heatmaps depicting top 50 enriched genes in 2D bronchioalveolar-like air–liquid cultures (2D ALI) compared to 3D lung bud tip organoids (3D LBT). $N = 2$ of 3D LBT, airway organoids on transwell system with apical medium (2D non-ALI), and 2D ALI. Colored bars represent Z-scores of $\log_2$-transformed values.

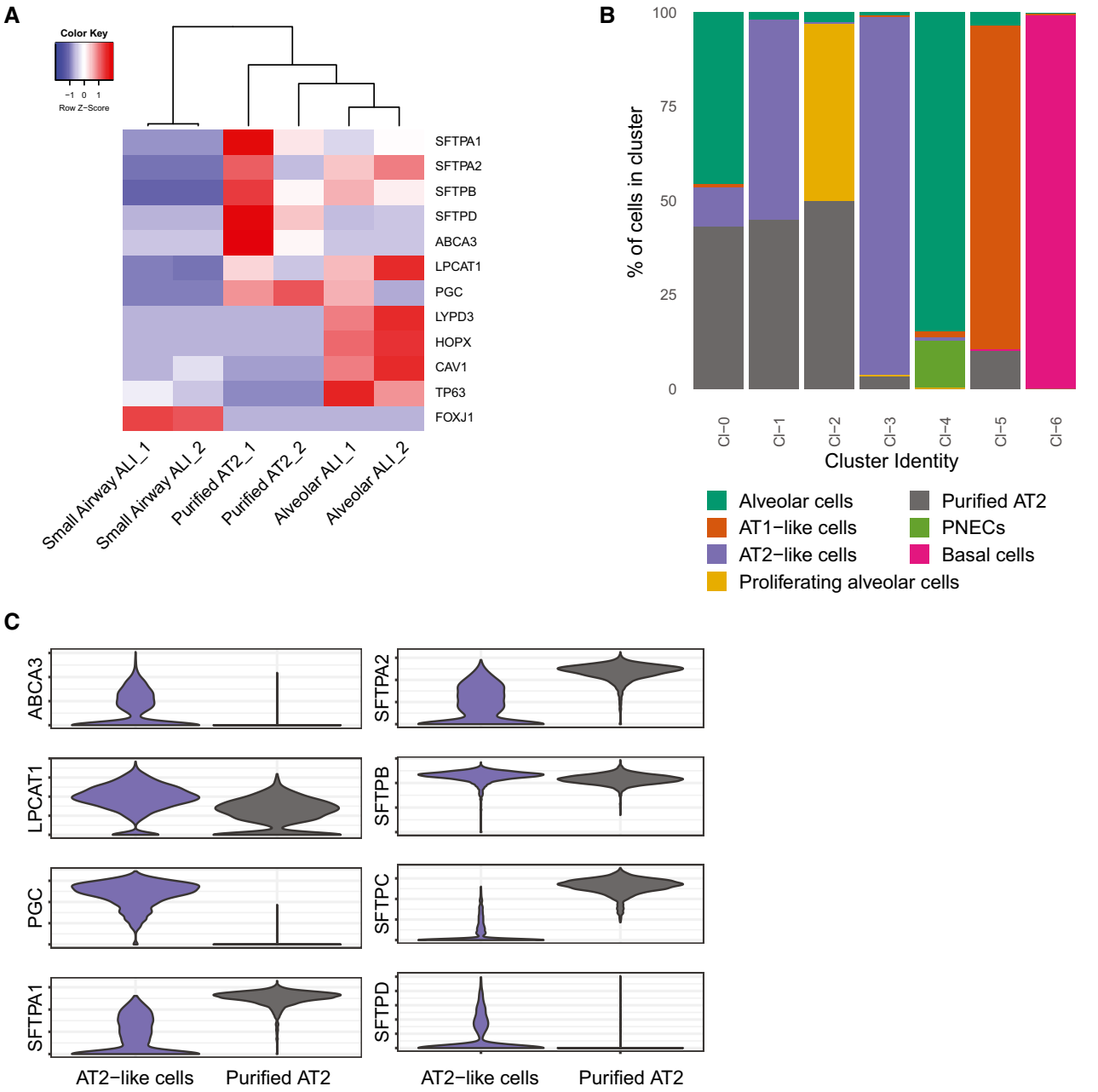

Figure EV3. Comparison of bronchioalveolar culture to published datasets of AT2 cells.

A Heatmaps depicting marker genes in 2D small airway air–liquid cultures (small airway ALI), bronchioalveolar-like air–liquid cultures (alveolar ALI), and published dataset of purified AT2 cells (purified AT2). Colored bars represent *Z*-scores of $\log_2$-transformed values.

B Barplot indicating percentage of annotated cells within a cluster. Color represents annotated cell identity. Combined current dataset and of GSM2855485 are presented. Indicated annotation is based on separate clustering. Published dataset GSM2855485 is annotated as "Purified AT2".

C Violin plots visualizing expression levels of marker genes for AT2 cells. AT2-like cells comprise cells annotated as AT2-like cells in current dataset (clusters 0 and 2). Purified AT2 cells are from published dataset GSM2855485.

Data information: Error bars represent SEM. $N = 4$ for donor 1 and $N = 3$ for donor 2. H p.i. = hours post-infection.

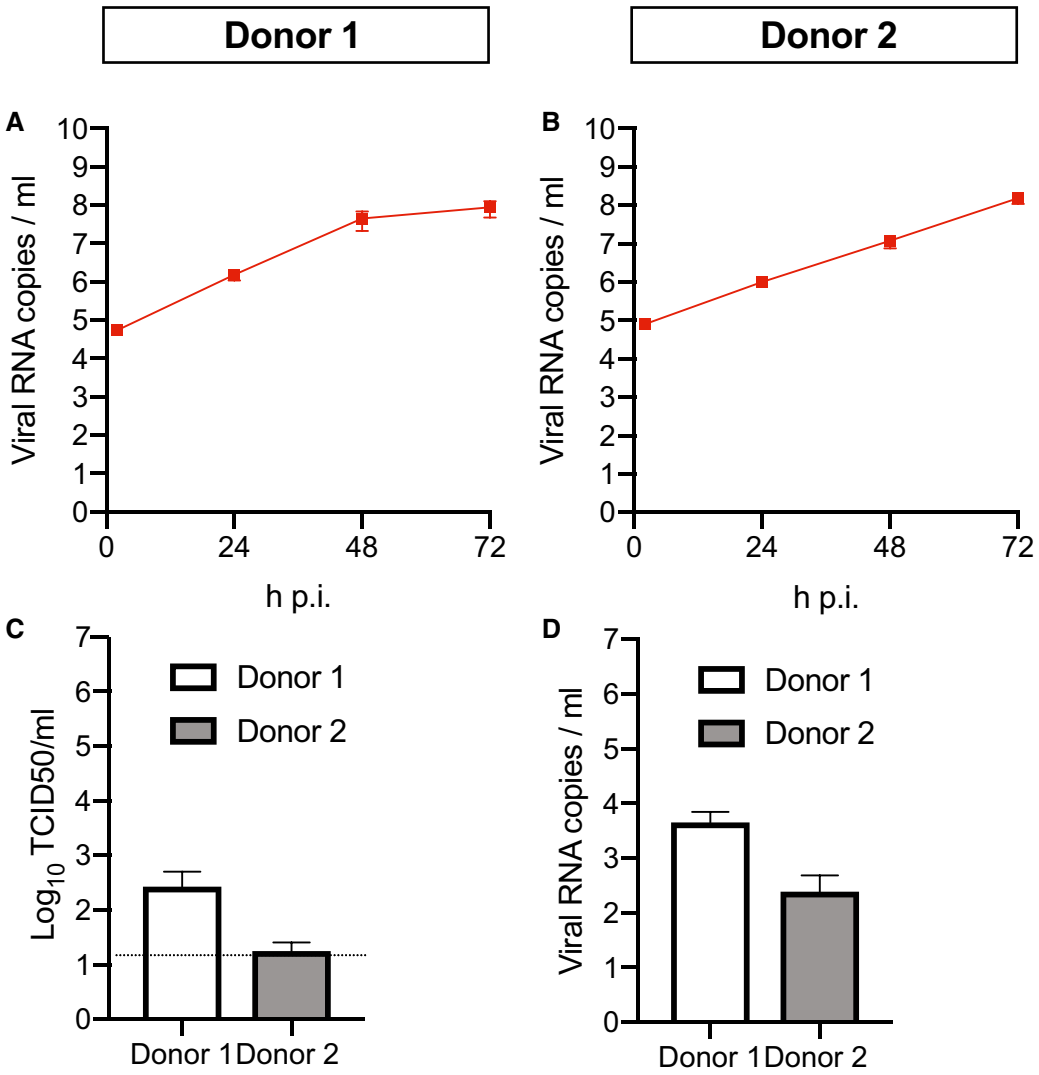

Figure EV4. Apical viral RNA and basal shedding in differentiated 2D bronchioalveolar-like cultures.

A, B Viral SARS-CoV-2 RNA titers of apical washes at 2, 24, 48, and 72 h after infection at MOI 0.1 for donor 1 (A) and donor 2 (B).

C, D Infectious virus titers as observed by virus titrations on VeroE6 cells (C) and qRT–PCR (D) in basal compartments. The dotted line indicates the lower limit of detection.

Data information: Error bars represent SEM. $N = 4$ for donor 1 and $N = 3$ for donor 2. H p.i. = hours post-infection.

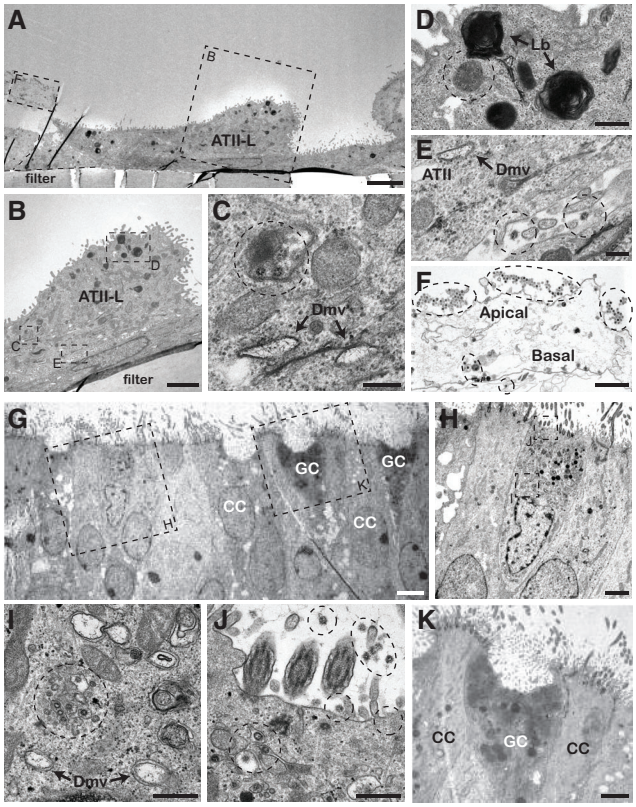

Figure EV5. Transmission electron microscopy analysis of 2D bronchioalveolar-like (A–F) and 2D small airway (G–K) cultures after 72 h of SARS-CoV-2 infection.

A–F Pulmonary alveolar type II-like (ATII-L) cells were found in the 2D bronchioalveolar-like culture (A, B). The early infection of the ATII-L cell (C) is recognizable by the double-membranes vesicles (Dmv) and virus particles (circles). (D) Next to the lamellar bodies (Lb), the ATII-L also contained a lysosomal compartment with internal virus particles (circle). Extracellular virus particles were found in the intercellular space between the ATI-L and ATII-L cells (E), albeit at low concentration. Dashed circles indicate viral particles outside of the cell. The remnants of a dead detached cell (F) mainly showed apical-shed virus particles (in areas marked by dotted lines), whereas the basal side was relatively free of virus particles.

G–K Ciliated cells (CC; (H)) and goblet cells (GC; (K)) are visible in the cross section through the 2D small airway culture after 8 weeks of differentiation. SARS-CoV-2 replication (I) and virus shedding (J) were found to be associated particularly with ciliated cells, whereas no infections were found in goblet cells (K). Dashed circles indicate viral particles inside and outside the cell.

Data information: Scale bars represent 5 μm (A, G), 2.5 μm (B, H, K), 1 μm (F), 500 nm (D, I, J), and 250 nm (C, E).