

Supplemental information

Methods

Western blot

For Western blot analysis, organoids were collected in RIPA Buffer (Cell Signaling Technology), supplemented with protease inhibitors (Complete Protease Inhibitor Cocktail Tablets, Roche Diagnostics, Switzerland). The amount of total protein was determined with Pierce BCA-Protein Assay Kit (Thermo Fisher, USA). Samples were denaturated and separated on a 12% SDS polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane, and membranes were probed against p21 (p21 (1/1000 diluted, Waf1/Cip1). The blots were stripped and reprobed for β -actin (1/1000 diluted, Nr. 4967, Cell Signaling Technology). Signals were detected by enhanced chemiluminescence (BioRad, Dreieich, Germany) using appropriate peroxidase-conjugated secondary antibodies (Agilent, DAKO, Santa Clara, CA, USA).

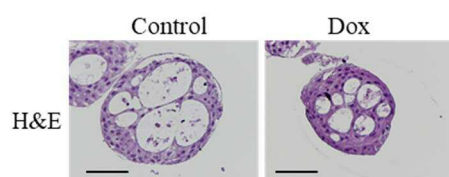
Histology/microscopy

Organoids from each group were pooled and embedded in paraffin as described before [1]. Sections of 2 μ m thickness were stained with hematoxylin-eosin (H&E). Deparaffinized paraffin sections were treated with BSA (1%) and Tween-20 in PBS (0.1%) and incubated overnight at 4 °C with the primary antibodies for p21 (1:100, Cell signaling (2947S)) and TIMP2 (1:400, Invitrogen (MA1-774)) in PBS containing BSA (1%). Cells were incubated for 30 min with secondary antibodies (goat anti-rabbit FITC, Sigma; goat anti-mouse, Cyanine 5, Thermo-Fisher Scientific). ImageJ software (National Institutes of Health) was used to analyze and merge the images.

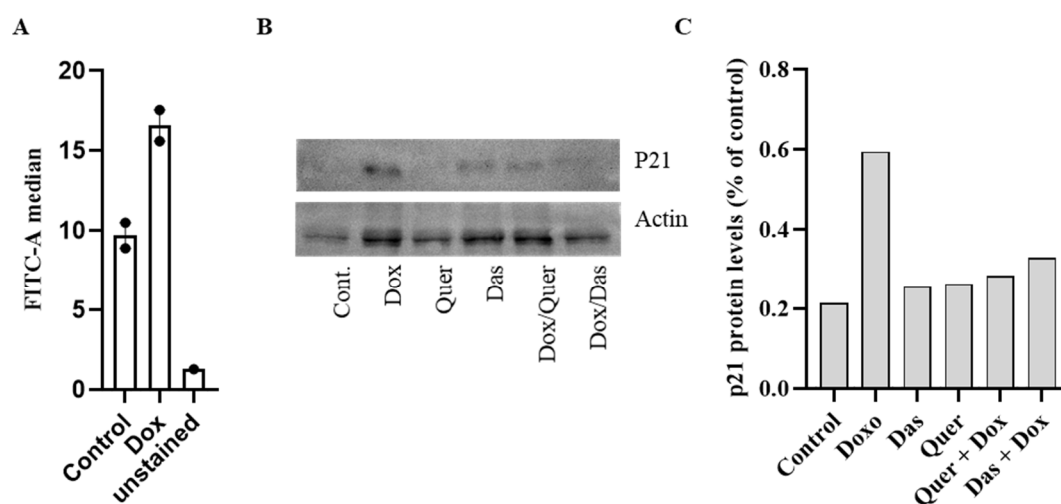
Senescence Assay (Flow Cytometry)

Organoids from two donors were treated with Dox or control media and dissociated as described in the single cell section. Activity of β -galactosidase was measured using the Senescence Assay Kit (Abcam, UK) according to the manufacturer's protocol.

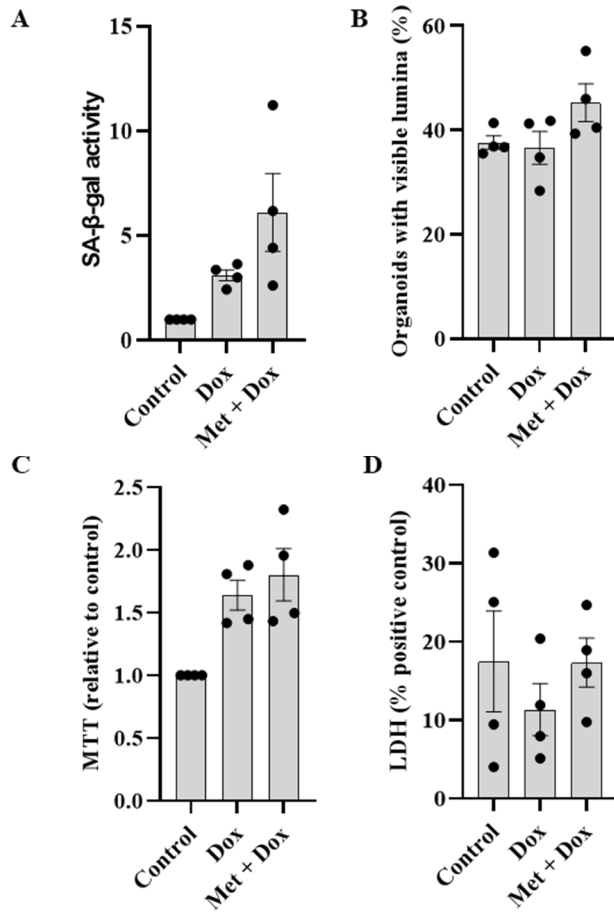
Supplementary figures



S1: Representative histology of embedded organoids (H&E staining, scale bar = 50 μ m).

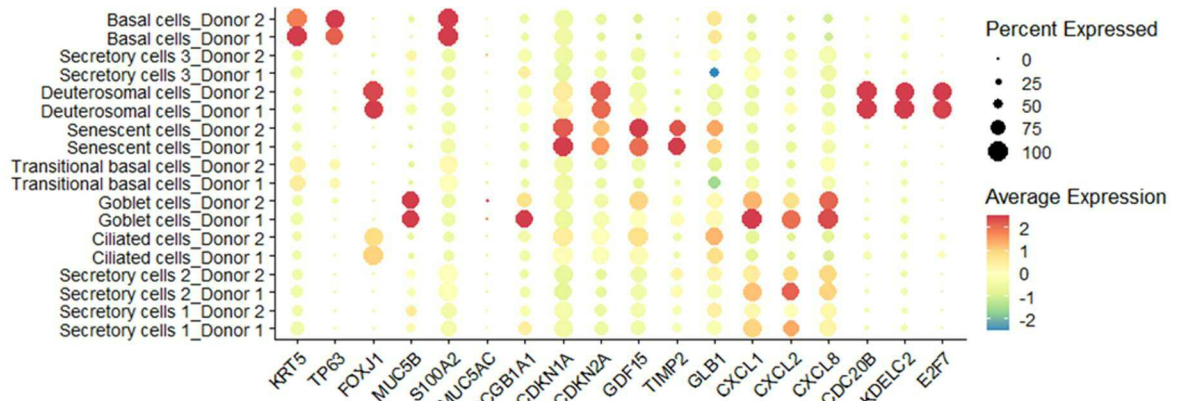


S2: (A) Organoids from two donors were treated with Dox for 48 hours and SA- β -gal activity was measured by flow cytometry. (B/C) Western blot analysis for p21. Organoids were treated with Dox and the combination of Dox with Quer or Das for 48 hours. (B) Cell lysates were gel separated and immunoblotted. (C) Semi-quantitative densitometry normalized to actin.



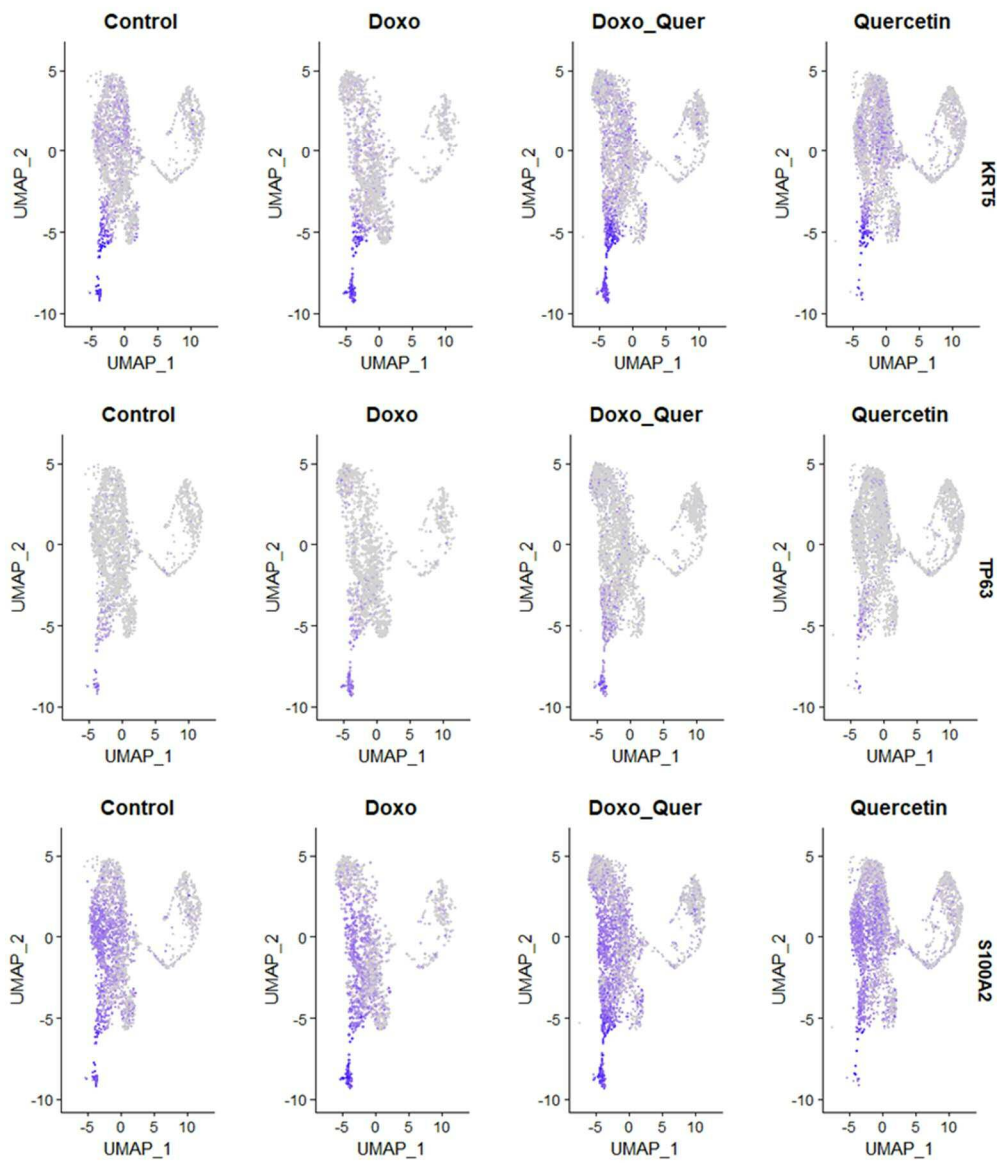
S3: Metformin does not inhibit senescence in organoids. (A) SA- β -gal activity, (B) percentage of organoids with visible lumen, (C) metabolic activity, and (D) release of LDH after 48 hours treatment of organoids with Dox and the combination of Dox with Met. Each data point represents an independent experiment. Data are shown as the mean $\pm$ SEM.

A

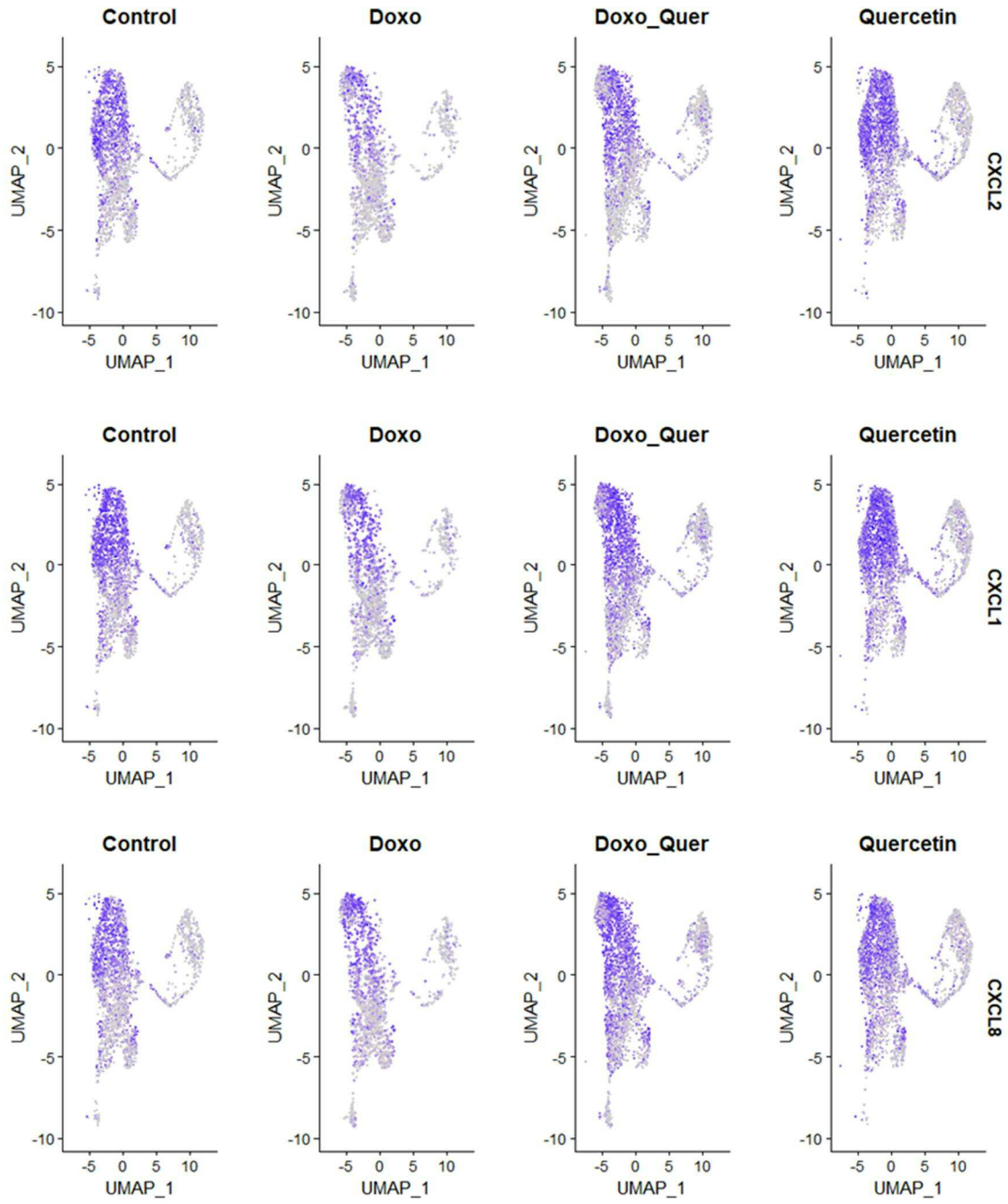


Basal cell marker

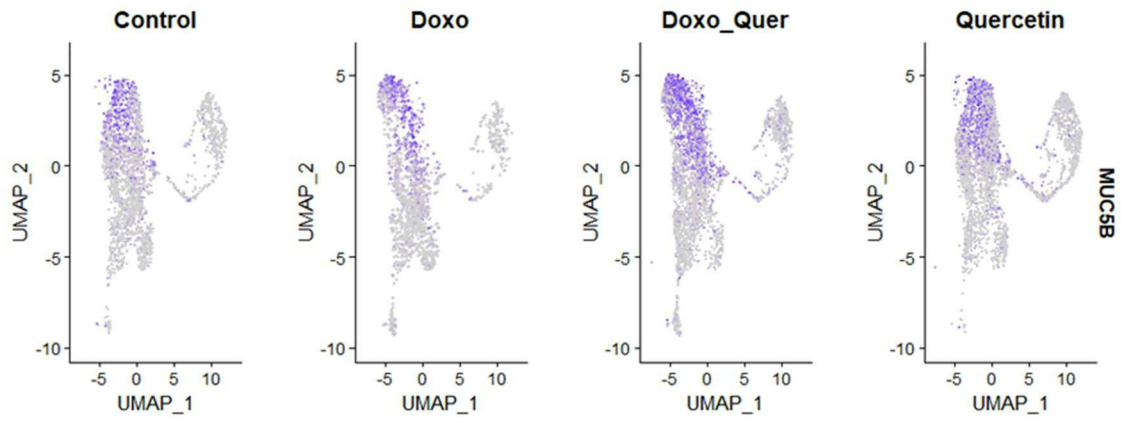
B



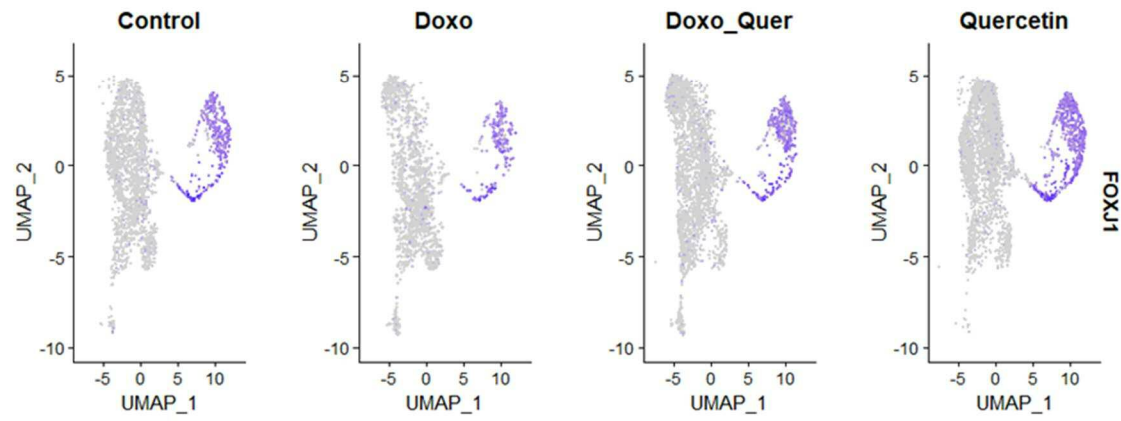
Secretory marker



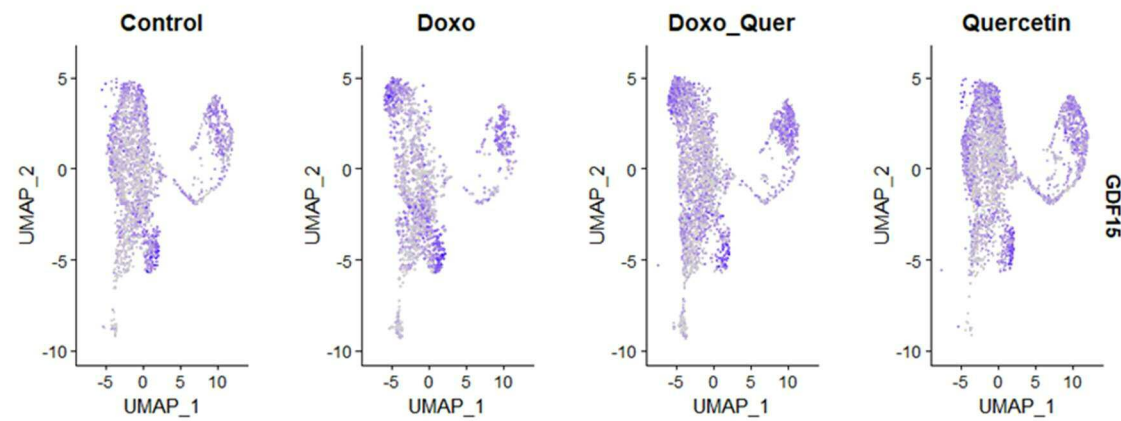
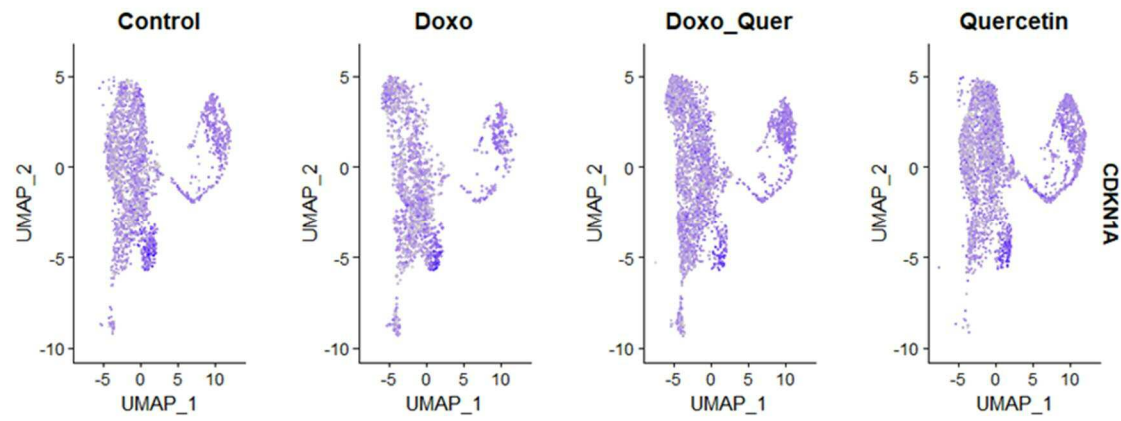
Goblet cell marker



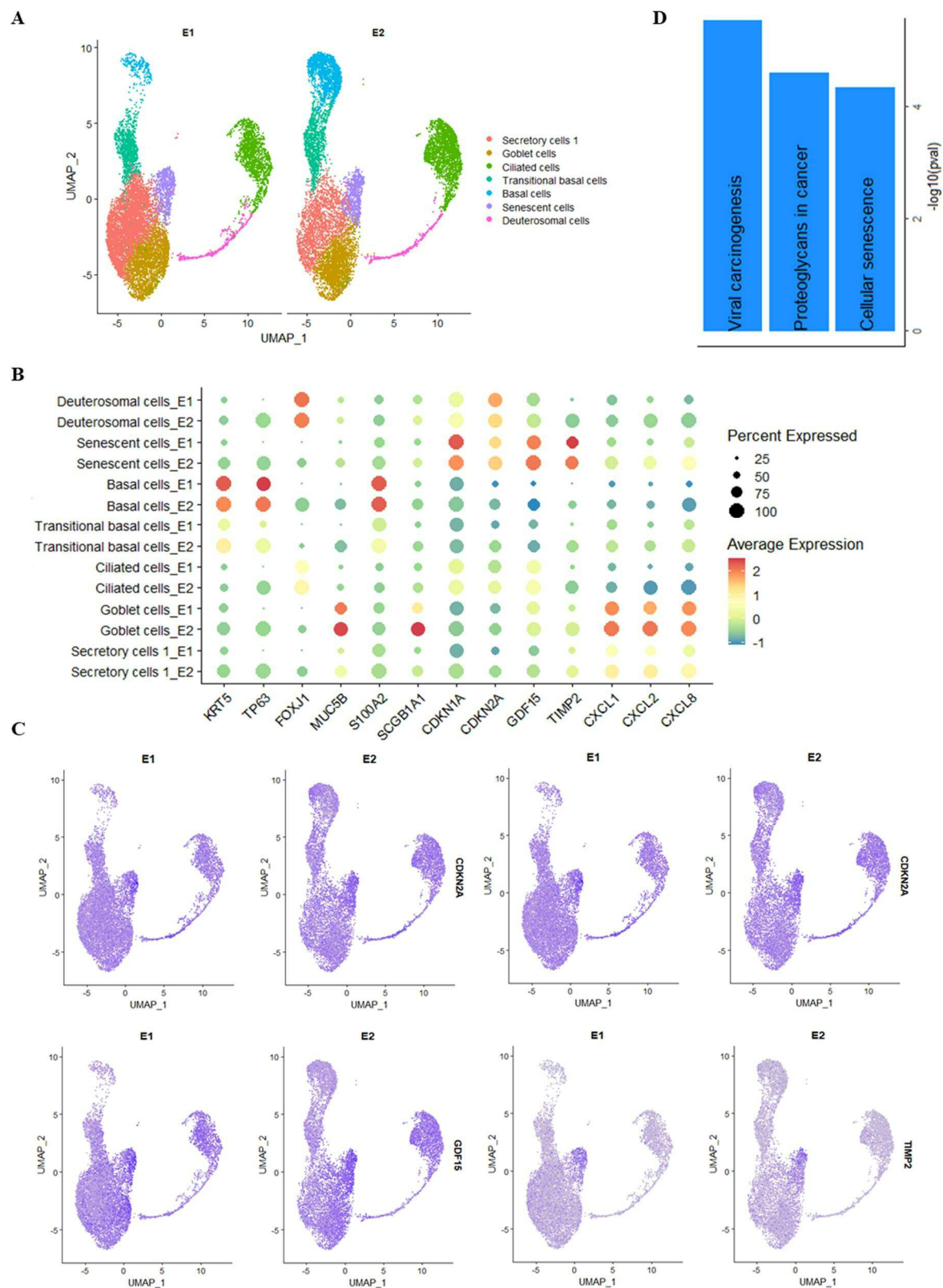
Ciliated cell marker



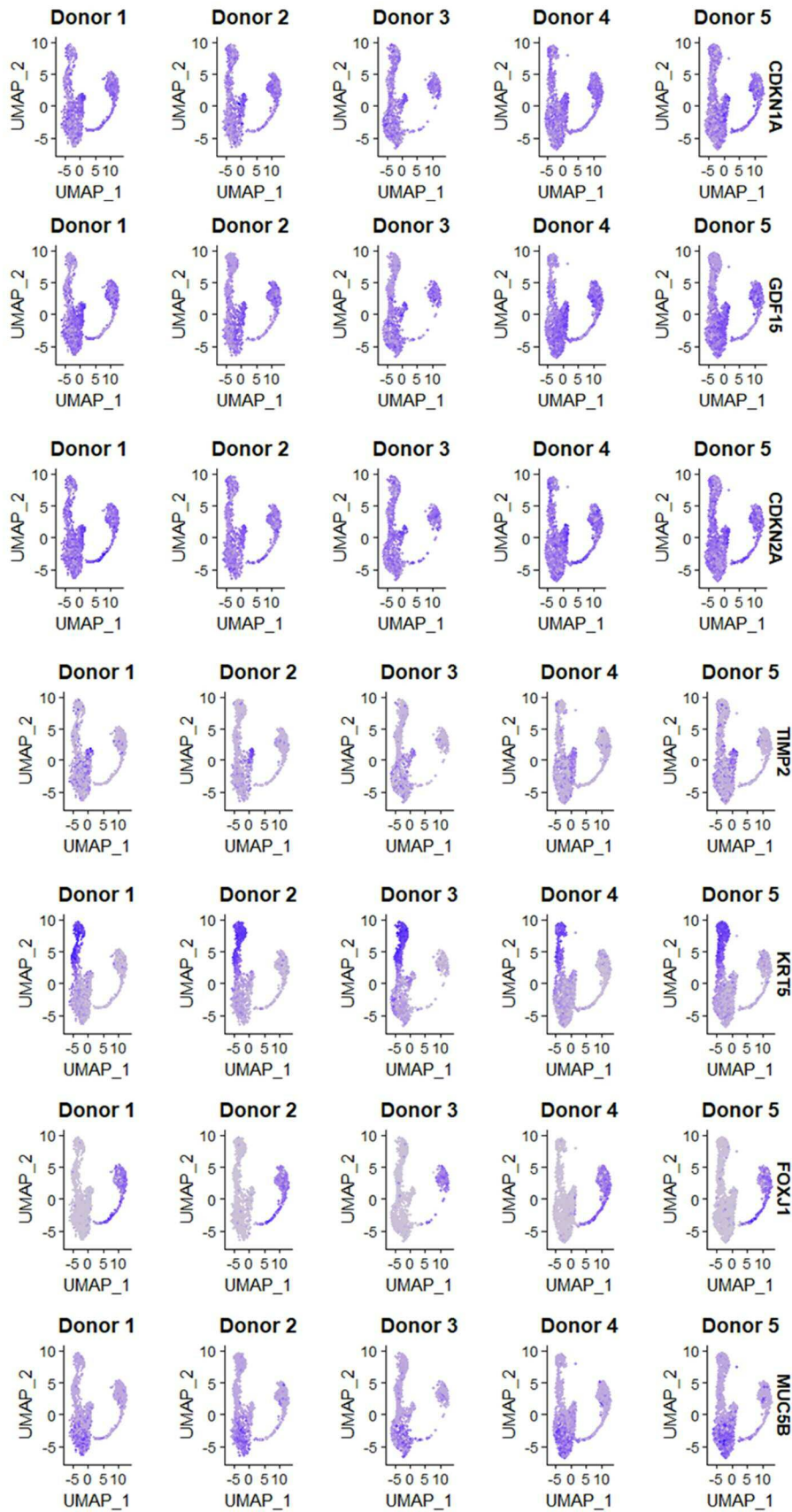
Senescence marker



S4: Organoids were pre-treated with Dox or control medium for 48 hours. The organoids pre-incubated with control medium were treated with control medium or Quer, the organoids pre-incubated with Dox were treated with Dox or the combination of Quer and Dox for additional 48 hours. (A) Dot plot showing expression of epithelial cell type markers. (B) UMAP visualization of cell markers.

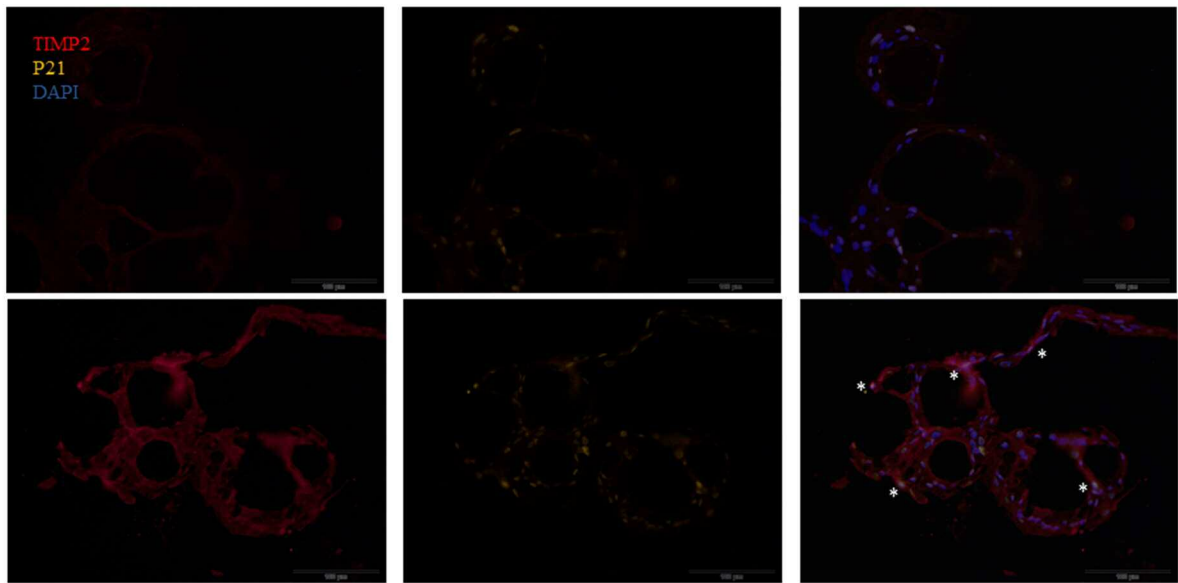


S5: The single cell data (E1) were combined with an independent data set containing untreated organoids from 5 donors (E2). (A) UMAP visualization of major epithelial cell types of combined data sets. (B) Dot plot showing expression of epithelial cell type markers. (C) UMAP visualization of cell markers.

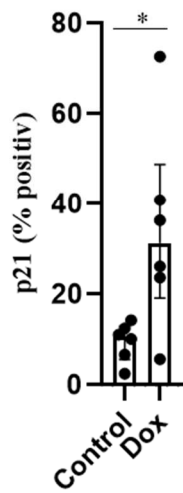


S6: The single cell data were combined with an independent data set containing untreated organoids from 5 donors. UMAP visualization of cell markers for the 5 donors

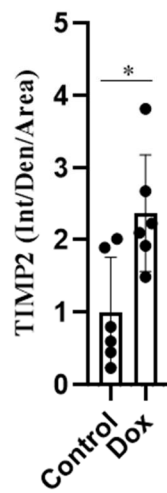
A



B

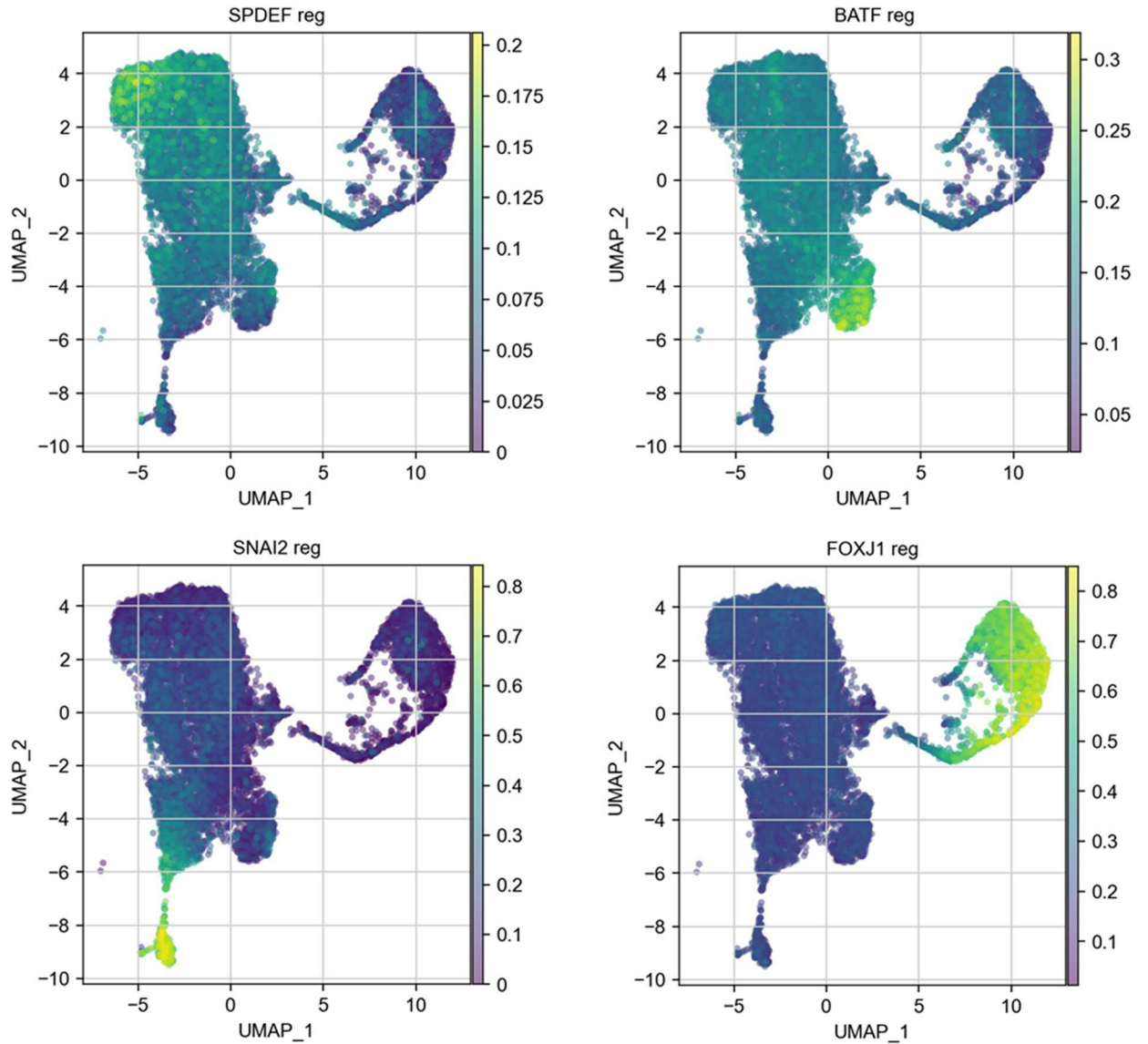


C



S7: Organoids were treated with Dox or control media for 48 hours. (A) Immunofluorescence staining was performed for TIMP2 (red) and p21 (green yellow). Nuclei were stained with DAPI (blue). Scale bar: 100 μ m. * indicate increased staining for TIMP2 associated with p21 positive nuclei. Quantification of (B) p21-positive nuclei and (C) TIMP2. Pooled results from two donors. Data were compared by Mann Whitney test and are shown as the median with interquartile range. $p < 0.05$.

B



S8: Organoids were pre-treated with Dox or control medium for 48 hours. The organoids pre-incubated with control medium were treated with control medium or Quer, the organoids pre-incubated with Dox were treated with Dox or the combination of Quer and Dox for additional 48 hours. (A) Heatmap of regulon activity of the top regulons discriminating the clusters. C, control media; D, Dox media; CQ, control media with Quer; DQ, Dox/Quer media. (C) UMAP representations.

1. Sprott RF, Ritzmann F, Langer F, Yao Y, Herr C, Kohl Y, Tschernig T, Bals R, Beisswenger C: **Flagellin shifts 3D bronchospheres towards mucus hyperproduction.** *Respir Res* 2020, **21**:222.