

Supplementary Figure, Table and Data Legends:

Supplementary Figure S1. The Impact of Donor Age and Duration from Collection-to-Processing on the Viability of Cells from Cryopreserved Tissue-Organoids.

A) Plot depicting viability percentages of bulk salivary cells post tissue-organoid dissociation across donor age (in years). **B)** Plot illustrating the percent viable salivary cells after tissue-organoid dissociation by duration of collection-to-processing of tissues (in hours).

Supplementary Figure S2. Analysis for Biomarker Expression in Male and Female Salivary Cells by FACS.

Plot showing percentage expression of various biomarkers (CD49f, PSMA, CD24, EpCAM, SSTR2, CD44, CD34) by flow cytometry in salivary cells of male and female donors (n=6).

Supplementary Figure S3. Comprehensive Immunophenotypic Analysis of Salivary Cells.

A) Salivary cells underwent immunophenotypic analysis using the antibody cocktail detailed in **Figure 2A**, with data analyzed using FlowJo. FACS data from six independent experiments were concatenated, normalized, and subjected to clustering for biomarker distribution analysis via t-SNE analysis. t-SNE plot showing expression of PSMA, EpCAM, CD24, and CD49f in salivary cells. Coloring correlates with expression intensity. Dark grey indicates high expression, while light grey signifies the absence of the cell marker expression. **B)** Barplot showing the expression of biomarkers by FACS across various passages. * indicate p-value < 0.05.

Supplementary Figure S4. Comparative Analysis of Biomarker Expression and Organoid Growth in Distinct Media Formulations.

A) Dynamic changes in biomarker expression profiles of unsorted (bulk) cells embedded in Matrigel and cultured in media-1 and media-2 across three sequential passages (P1-P3). **B)** Comparative analysis of biomarker expression profiles within the 3D OIC assay between media-1 and media-2. **C)** Comparison between matched patients (n=5) based on organoids generated in media-1 and media-2, categorized by distinct phenotypes seeded. **D)** Representative brightfield images of cultured SMG and PG cells at Passage 3 in media 1 and 2 (200x magnification). **E)** Representative immunofluorescence images of cultured PG cells stained for DAPI (blue) to indicate nuclei and E-cadherin (green) as a marker of epithelial cell-cell junctions. The merged image illustrates the spatial localization and overlap of nuclei and E-cadherin staining (100x magnification). Statistical analyses are denoted within the graphs, where 'ns' signifies non-significant differences and * p-value < 0.05 and **** p-value < 0.0001.

Supplementary Figure S5. Cell Output Analysis from Single Cells. Plots showing cell output per starting single cells obtained from **A)** SMG **B-C)** PG cells cultured with or without collagen in media-1 or N2 media.

Supplementary Figure S6. Microscopic Analysis of 3D-Matrigel Organoids. **A)** Brightfield images (4x and 10x) of 3D-matrigel organoids obtained from FACS-enriched (CD24+PSMA-, CD24+PSMA+, EpCAM+CD49f+, EpCAM+CD49f-, CD49f+, and CD49f-) of salivary cell subpopulations (Scale 1000µM and 200µM).

Supplementary Figure S7. Correlation of Biomarker Expression in Salivary Cells by FACS. Plots showing the correlation between expression of CD49f, EpCAM and CD24 markers in **A)** PG and **B)** SMG cells.

Supplementary Figure S8. Biomarker Profiles in PG and SMG Cells across Passages. Merged t-SNE clusters depicting the expression of CD49f, PSMA, CD24, EpCAM, SSTR2, CD34, CD44, and CD31/CD45 in **A)** PG and **B)** SMG cells across passages P0 and P1. Coloring corresponds to expression intensity, where red indicates high expression, and blue signifies the absence of the cell marker expression. **C)** Heatmap illustrating the expression of PSMA, CD49f, CD24, EpCAM and SSTR2 across passages P0 and P1.

Supplementary Figure S9. A Venn diagram showing number of shared and unique differentially expressed protein counts per cell within the progenitor-rich single cells of both SMG and PG in **A)** Run1 and **B)** Run2. **C)** Pie chart showing molecular type of proteins identified in each of the PG (N=3) and a SMG sample (N=1). **D)** Pie chart sub-cellular location of proteins identified in each of the PG (N=3) and a SMG sample (N=1).

Supplementary Figure S10. SGSPC-expressed Cytokeratins and their Pattern of Immunohistochemical Staining within Salivary Acinar Myoepithelial Cells (Source: Human Protein Atlas).

Supplementary Figure S11. Plots showing DCF fluorescence in PG cells (P1, N=2) following incubation with increasing concentration of hydrogen peroxide (0-50 µM) for 15 minutes.

Supplementary Figure S12. Uncropped agarose gel (2%) images showing expression of *AQP5*, *ACTB* *FOLH1* (*PSMA*) and *AMYL1A*, in 2D cells and 3D Matrigel organoids of PG and SMG cells across two consecutive passages.

Supplementary Table S1. Composition of Human Salivary Gland Tissue Dissociation Media.

Supplementary Table S2. Details of Antibodies Used for Immunohistochemistry.

Supplementary Table S3. Details of Antibodies Used for FACS Analysis.

Supplementary Table S4. Primer sequences used for RT-PCR

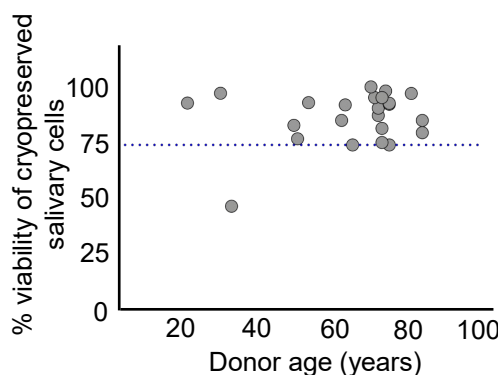
Supplementary Data 1. List of Proteins Identified from Single-cell Proteomics of Progenitor-Enriched Salivary Epithelial Cells.

Supplementary Data 2. Post-translational Modifications of Proteins Identified by Single-Cell Proteomics Analysis of Progenitor-Enriched Salivary Epithelial Cells.

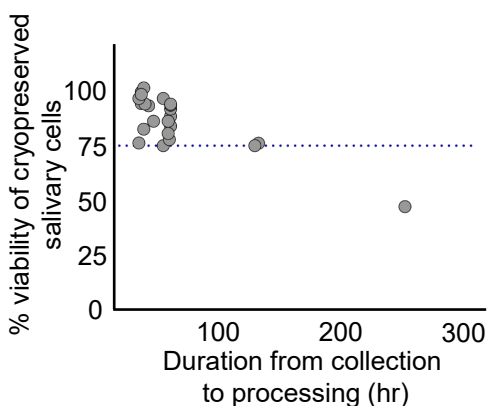
Supplementary Data 3. Batch effect Corrected Relative Abundance of Proteins Identified $\geq 50\%$ of Cell Types

Supplementary Figure S1

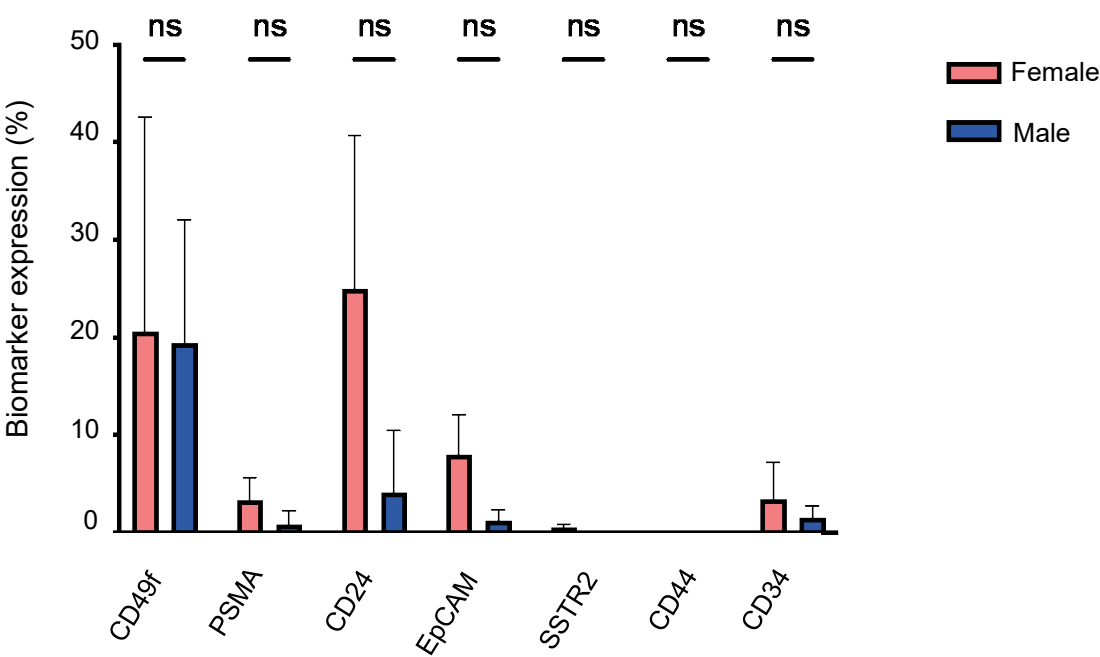
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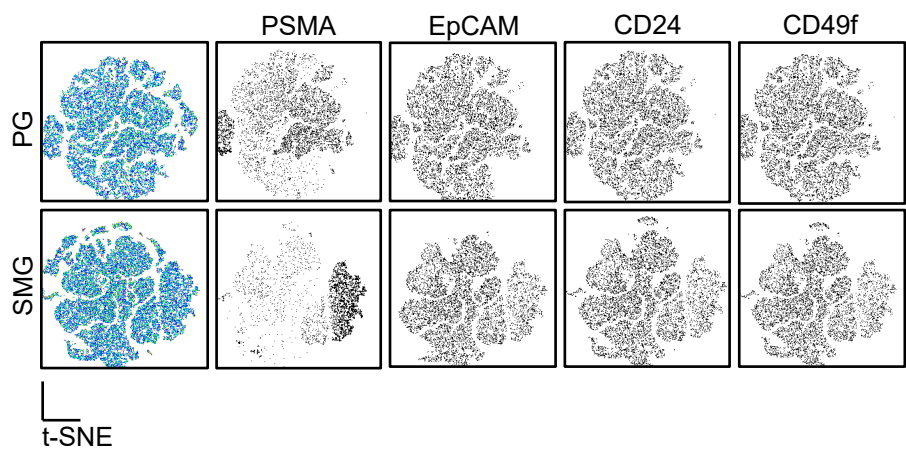


Supplementary Figure S2

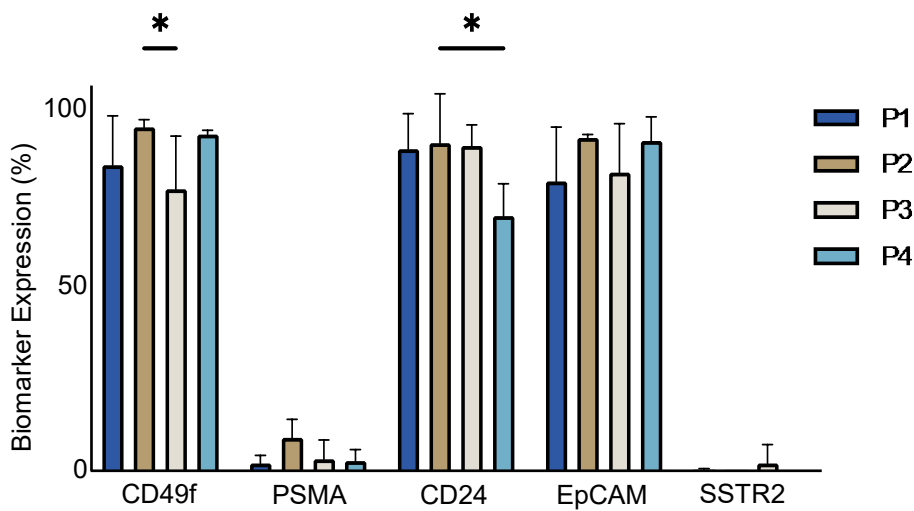


Supplementary Figure S3

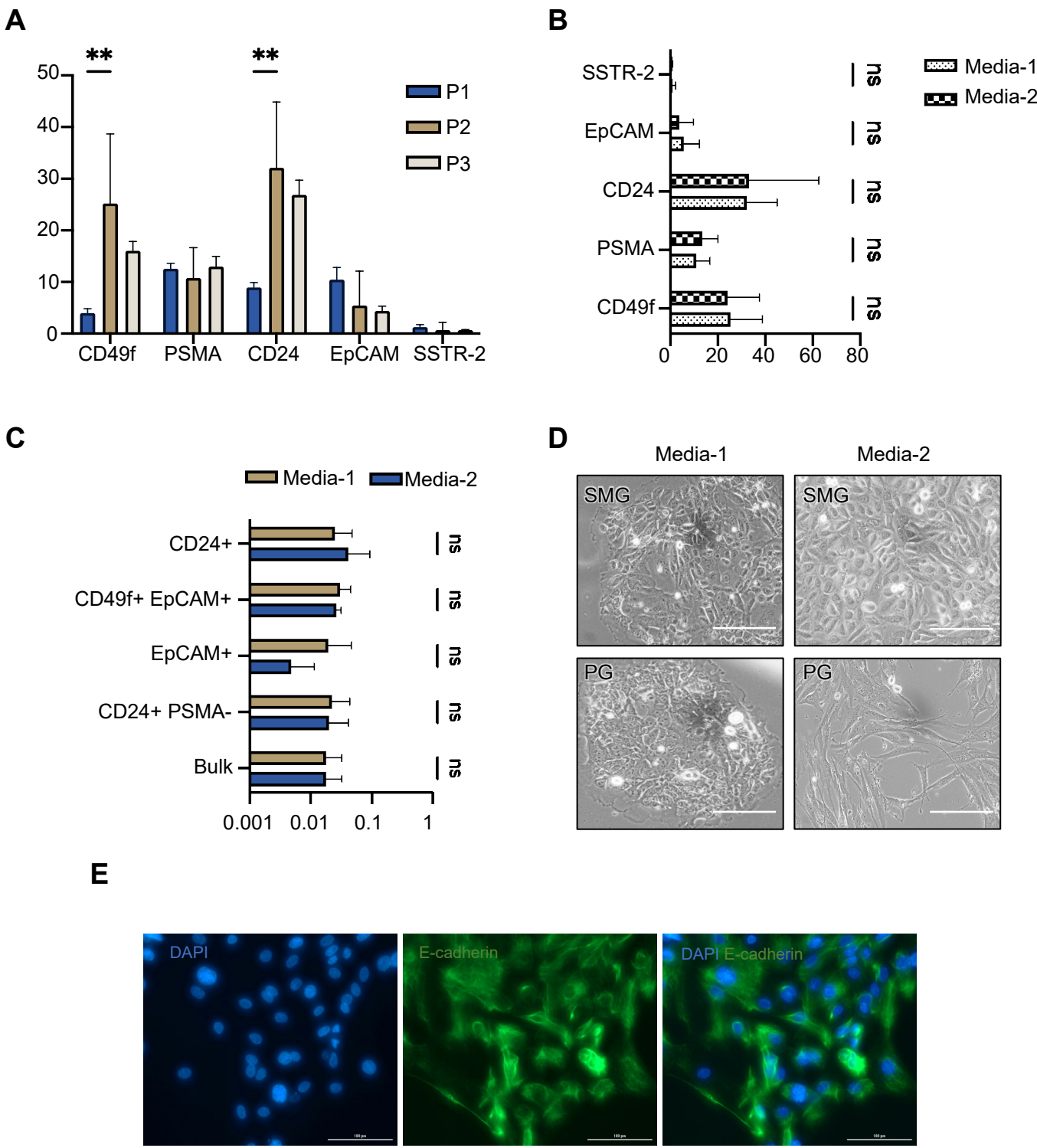
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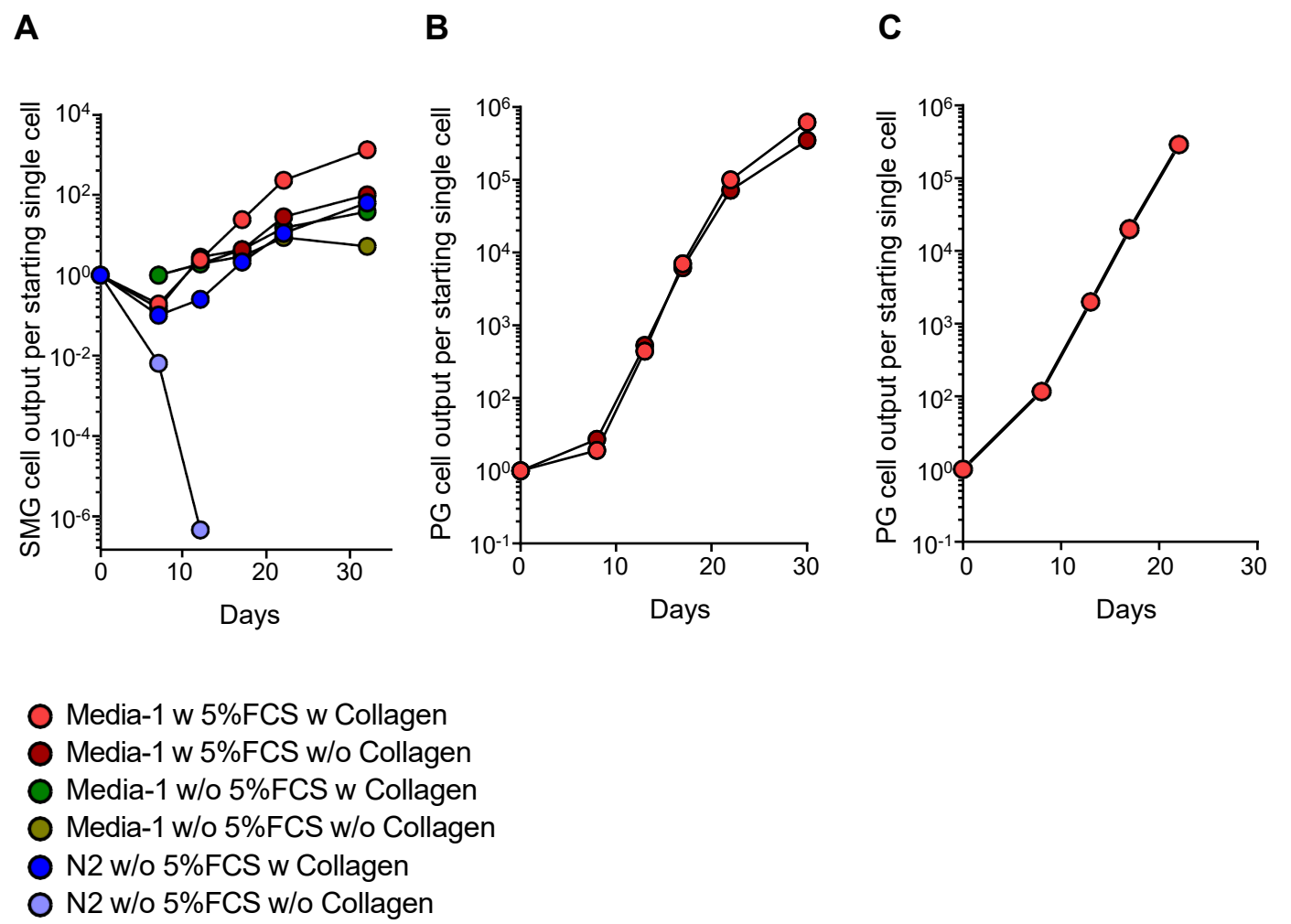
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Supplementary Figure S4

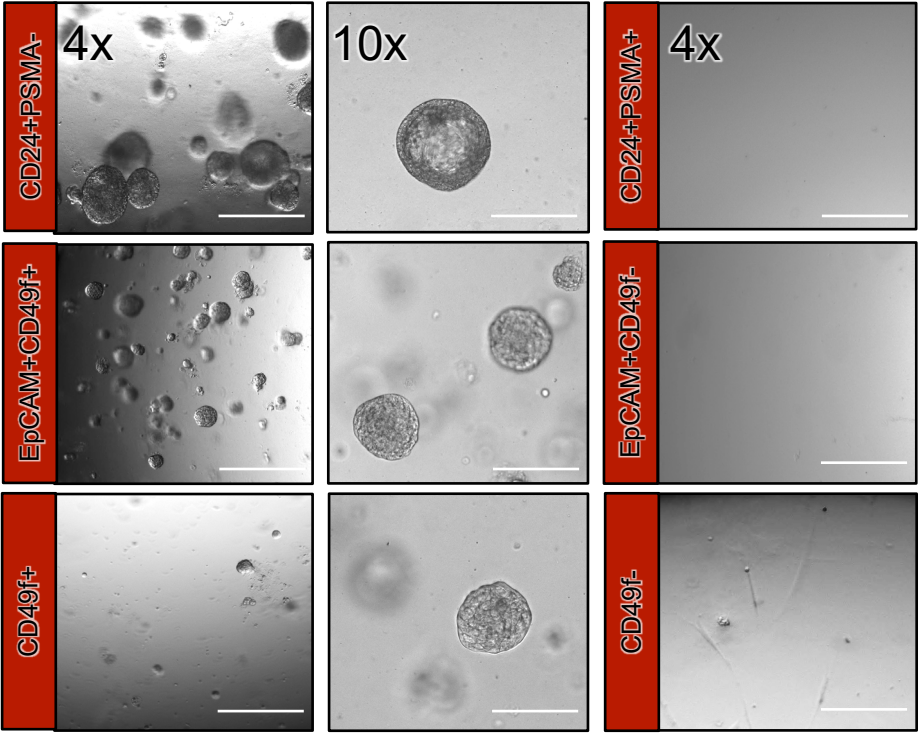


Supplementary Figure S5

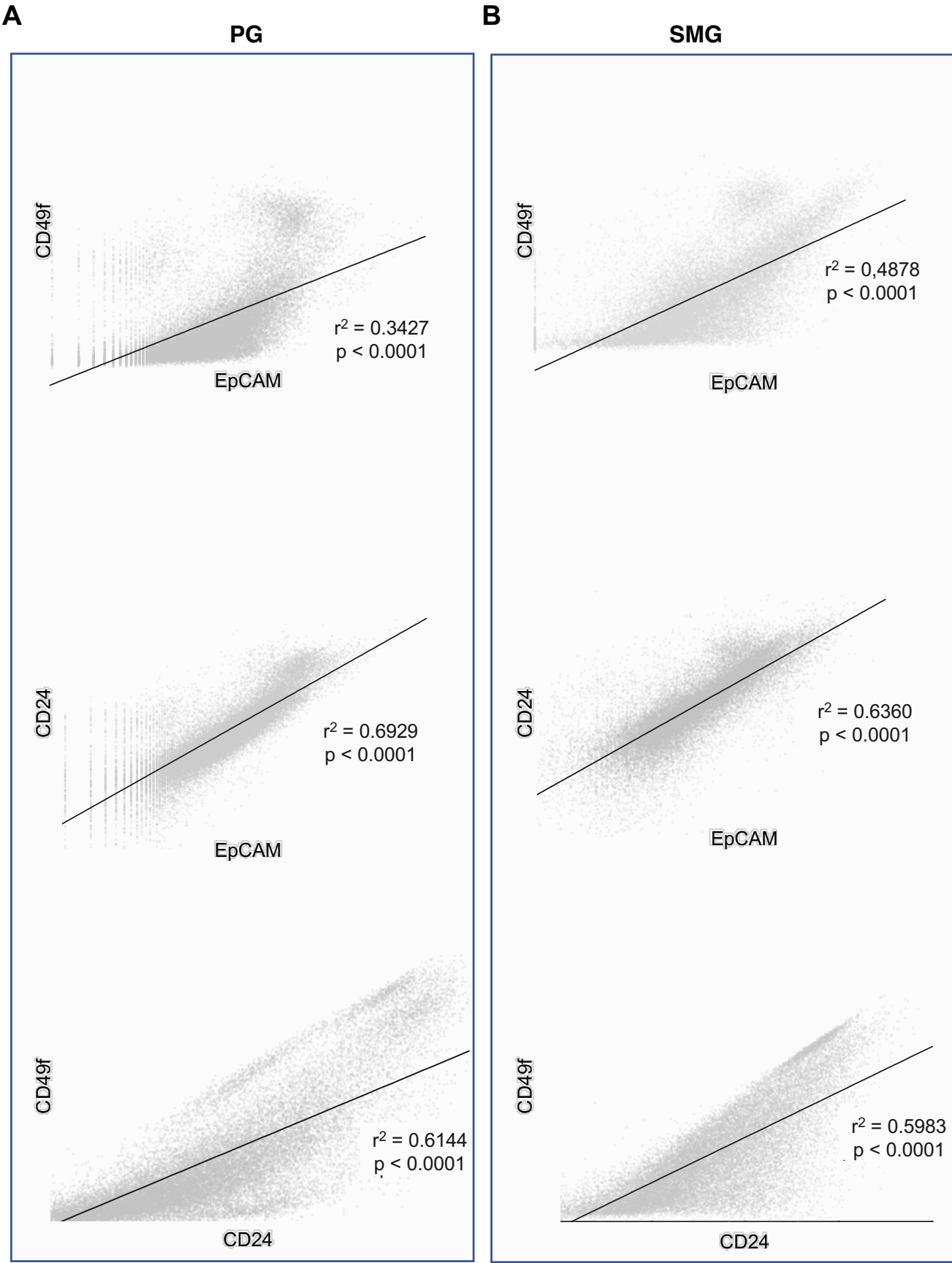


Supplementary Figure S6

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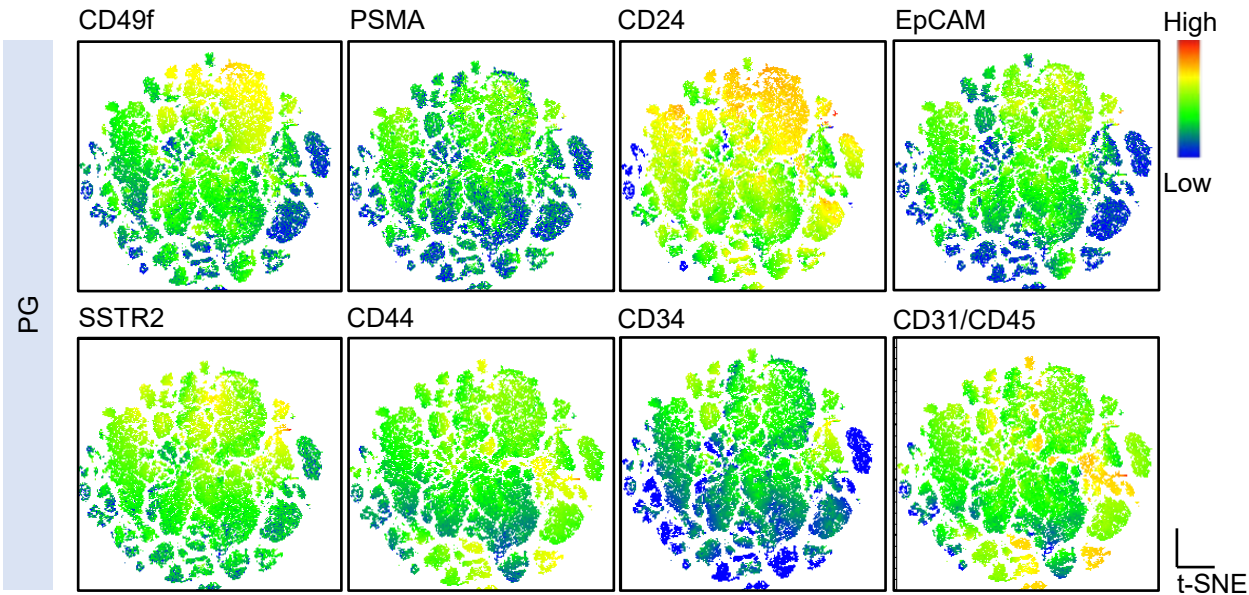


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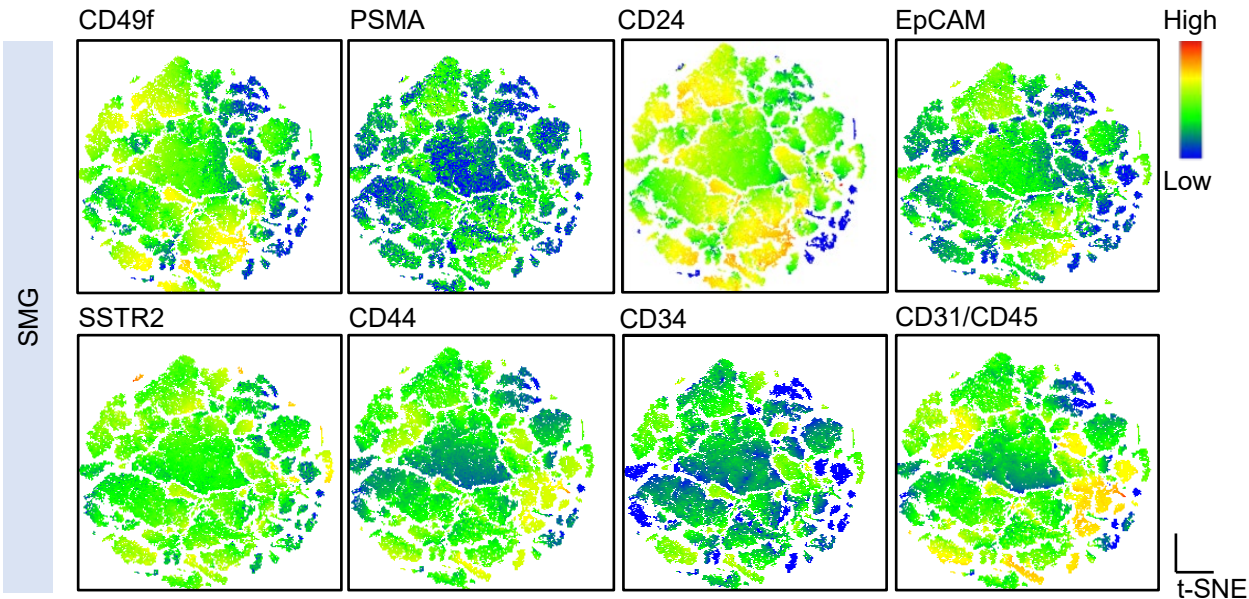


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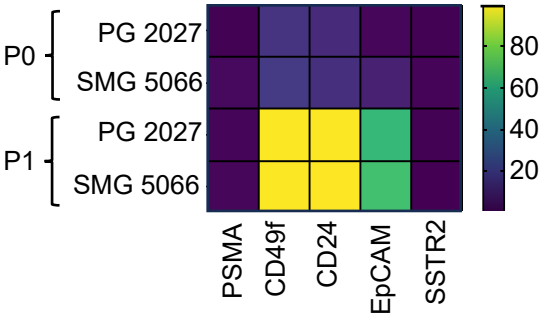
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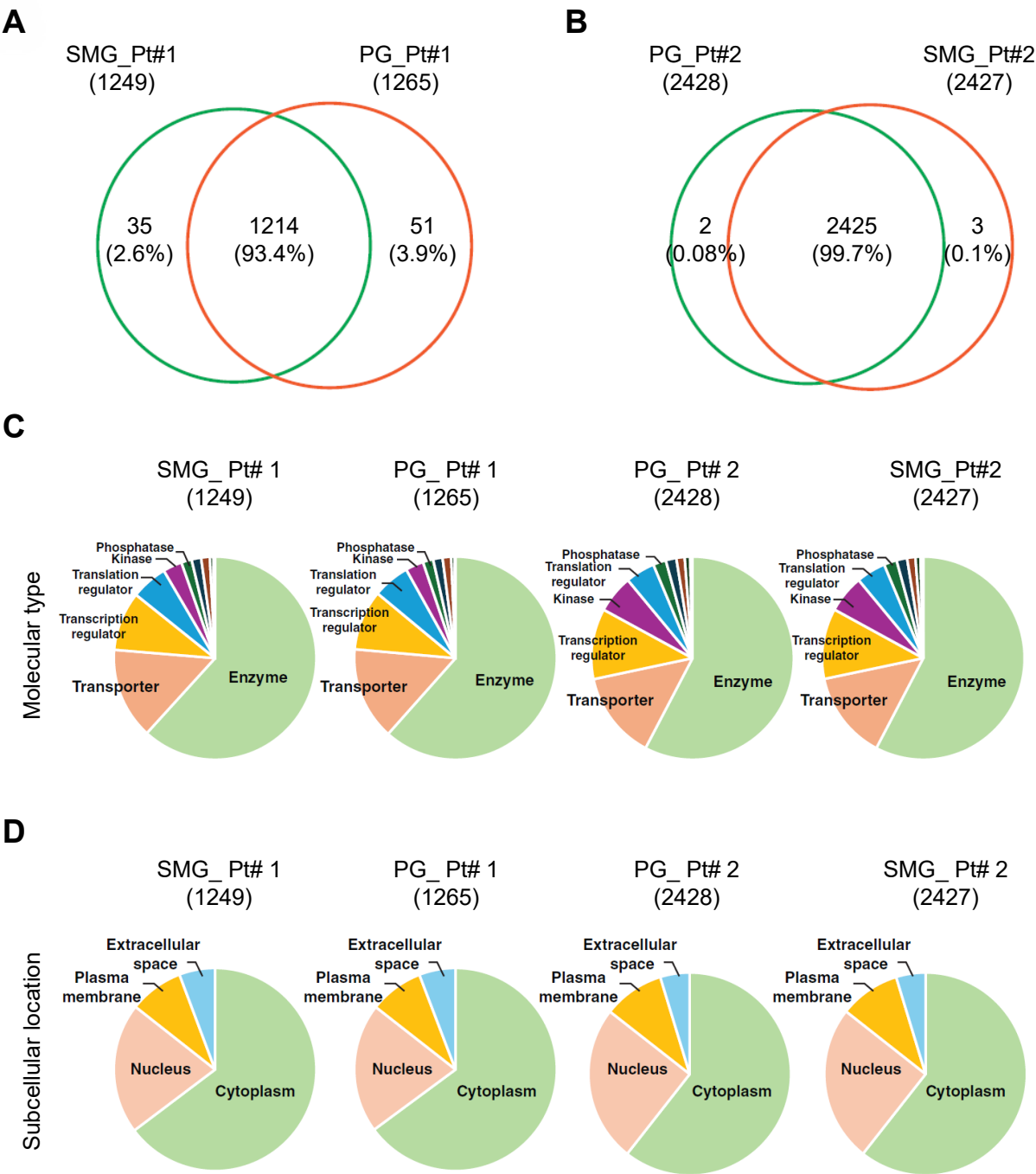
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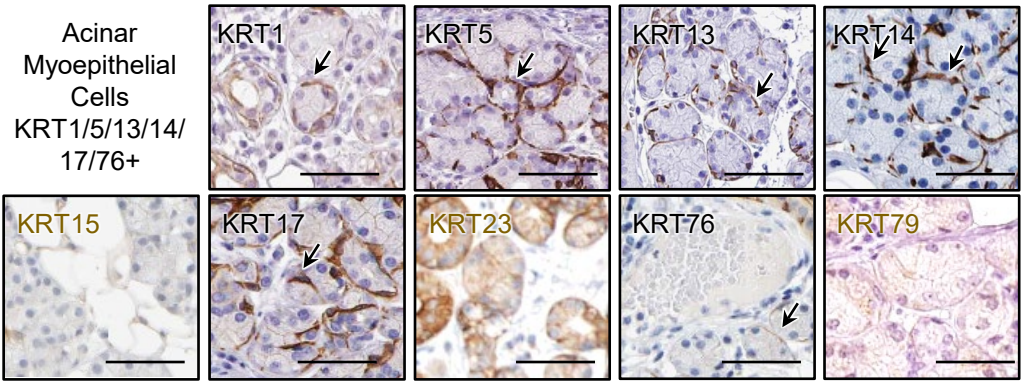
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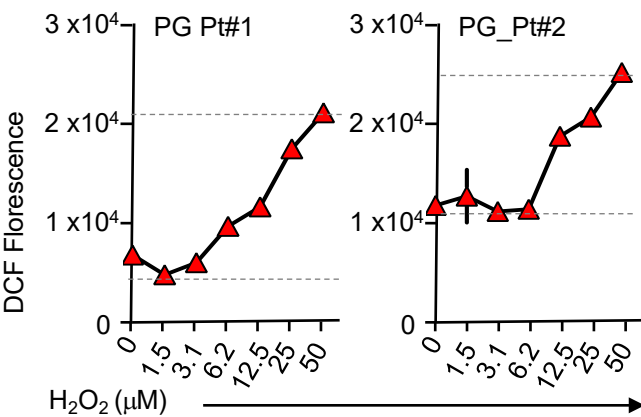
Supplementary Figure S9



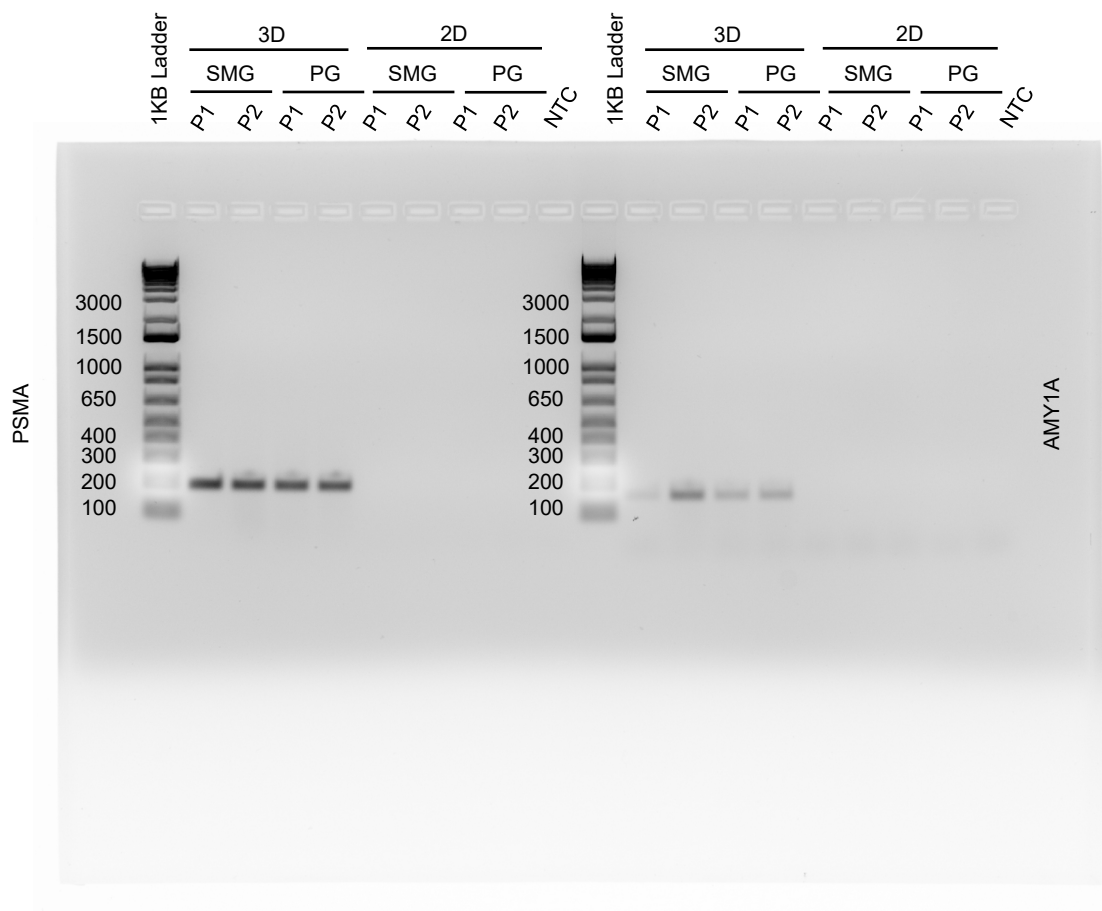
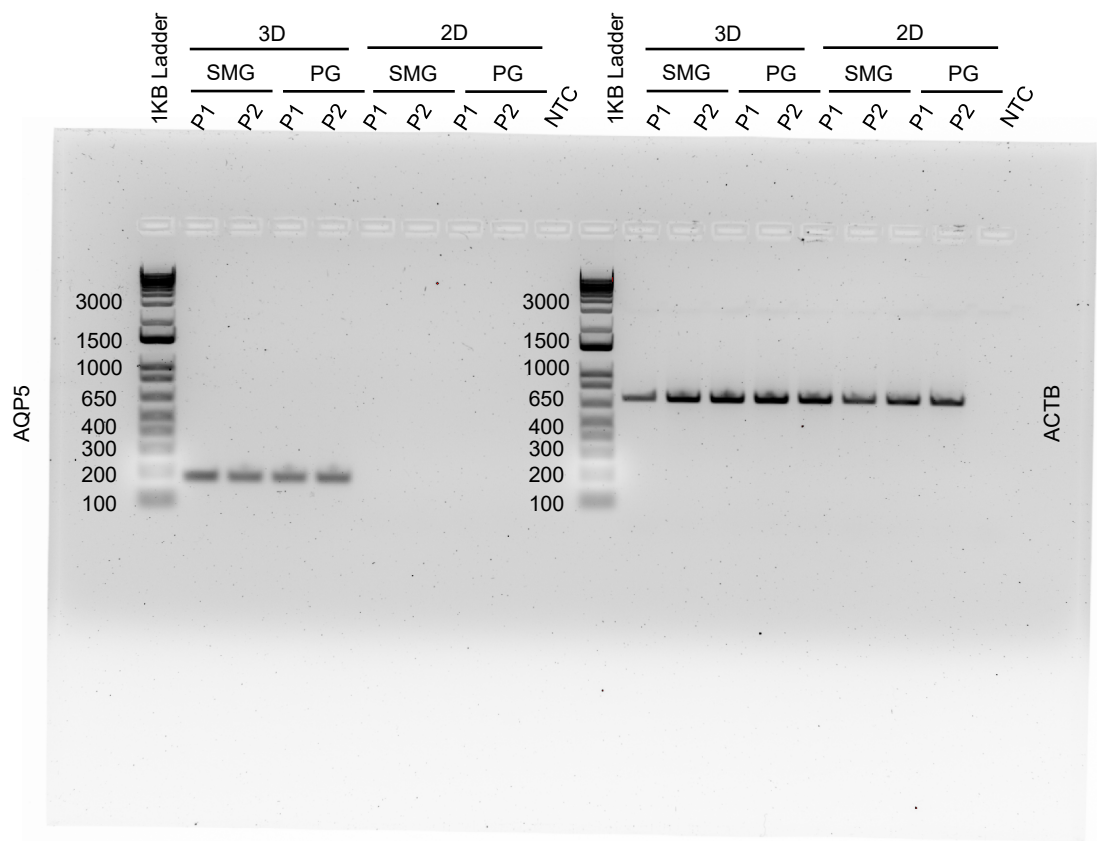
Supplementary Figure S10



Supplementary Figure S11



Supplementary Figure S12



Supplementary Table S1 – Composition of Human Salivary Gland Tissue Dissociation Media

Reagent	Vendor	Catalog Number	Stock Concentration	Final Concentration
DMEM-F12, Glutamax	Gibco	10565018	NA	1X
BSA	Sigma-Aldrich	A1470-100G	Powder	2% (W/V)
Collagenase (3000U/ml), Hyaluronidase (1000U/ml) mixture	Sigma-Aldrich	C9891-5G H3506-5G	10X	1X
Human recombinant insulin	Sigma-Aldrich	91077C-100MG	1 mg/ml	1µg/ml
Anti-Anti	Gibco	15240062	100X	1X

Supplementary Table S2: Details of antibodies used for immunohistochemistry

Primary and secondary antibodies	Dilution	Clone ID	Catalog #	Vendor
Anti-NKCC1	1:250	NA	ab59791	Abcam
Anti-Aquaporin-5	1:200	NA	AB15858	Millipore Sigma
Anti-Cytokeratin 5	1:500	RCK105	ab9021	Abcam
Anti-Cytokeratin 7	1:250	EP160Y	ab52635	Abcam
Anti-Cytokeratin 8	1:250	TROMA-1	MABT329	Millipore Sigma
Anti- Actin, Smooth Muscle	1:250	Ab-1(1A4)	MA1-06110	Thermo Fisher Scientific
Alexa Fluor® 488 goat anti-rabbit IgG	1:500	NA	A-11008	Thermo Fisher Scientific
Alexa Fluor® 594 goat anti-rabbit IgG	1:500	NA	A-11012	Thermo Fisher Scientific

Supplementary Table S3 – Details of the antibodies used for FACS analysis.

Antibody	Antibody Clone	Antibody Source	Flouochrome
Anti-PSMA	LNI-I7	BioLegend	PE-Cy7
Anti-EpCAM	9C4	BioLegend	PerCP-Cy5.5
Anti-SSTR2	402038	R&D Systems	PE
Anti-CD34	561	BioLegend	Alexa 488
Anti-CD44	515	BD Biosciences	BV510
Anti-CD31	WM59	BioLegend	PacBlue
Anti-CD45	HI30	BioLegend	PacBlue
Anti-CD24	ML5	BioLegend	APC-Cy7
Anti-CD49f	GoH3	R&D Systems	APC

Supplementary Table S4 - Primers used for RT-PCR analysis

Gene Name	Sequence	Amplicon Size
AMY1A_Foward	CAAATG AAAATG TTG CCA TTC AC	162 bp
AMY1A_Reverse	TCAATT CTG AAC CCT GCAACA C	
AQP5_Foward	TCAACAACAACA CAA CGC AG	178 bp
AQP5_Reverse	CCT ACC CAG AAAACT CCG AC	
FOLH1 (PSMA)_Forward	CAT GCC CAG GAAACA AAC AA	180 bp
FOLH1 (PSMA)_Reverse	TCG ACAATC AAAAGG GAG CA	
ACTB_Foward	CCT GGA CTT CGA GCAAGA GAG	494 bp
ACTB_Reverse	CTA ACG CAA CTAAGT CAT AGT CCG	