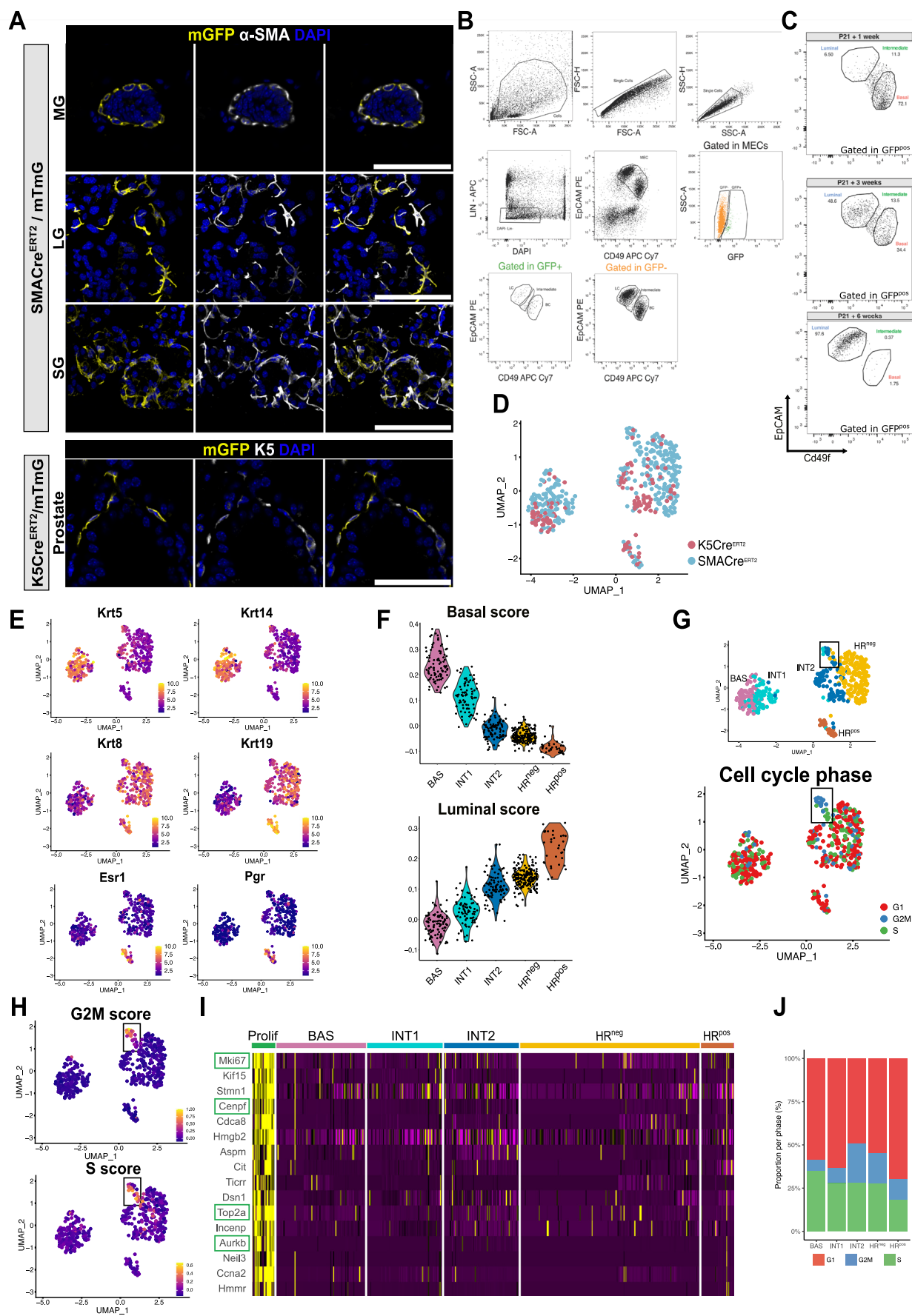
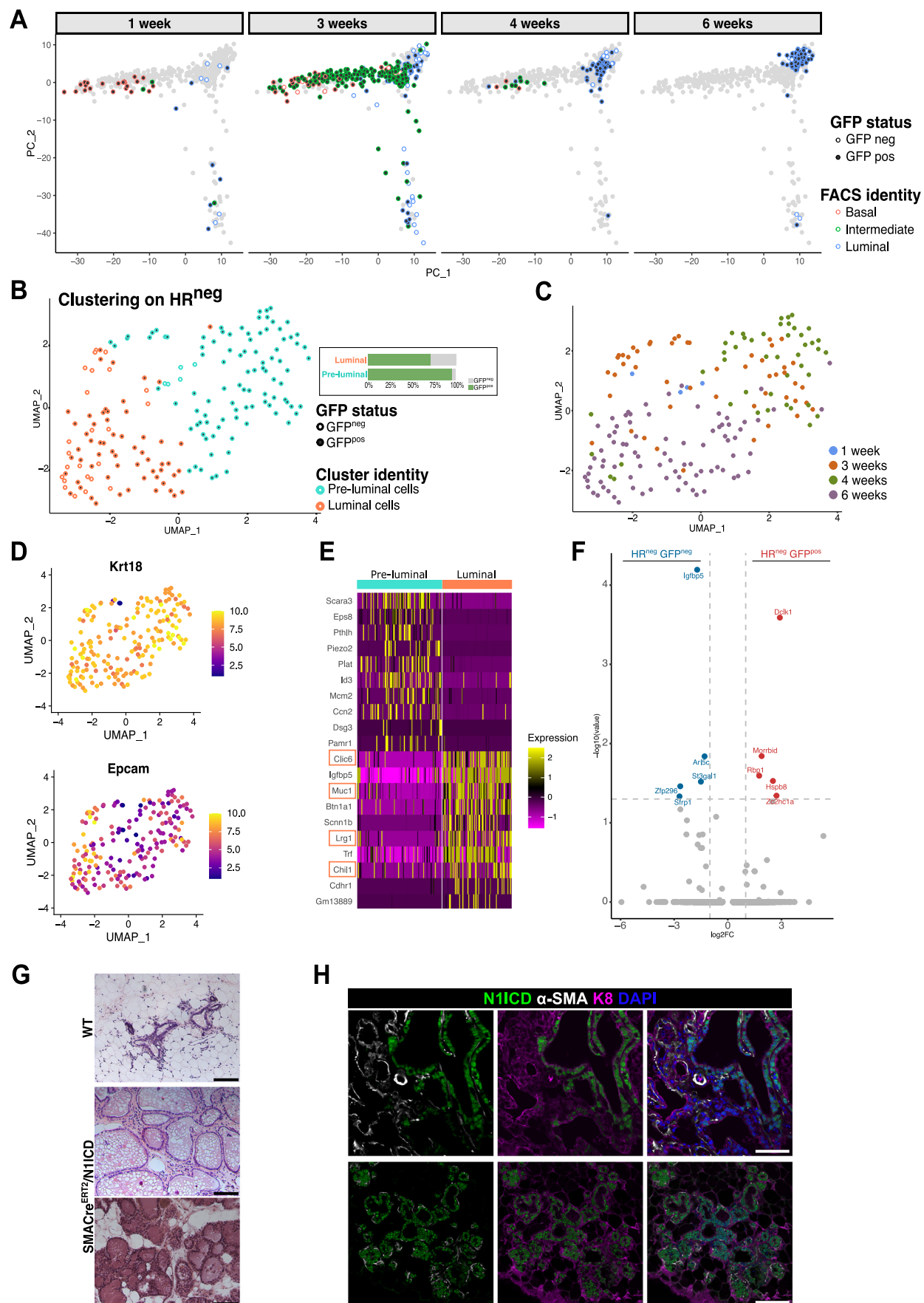


Expanded View Figures

Figure EV1. Transcriptional signatures characterizing the different cell clusters identified by UMAP analysis.

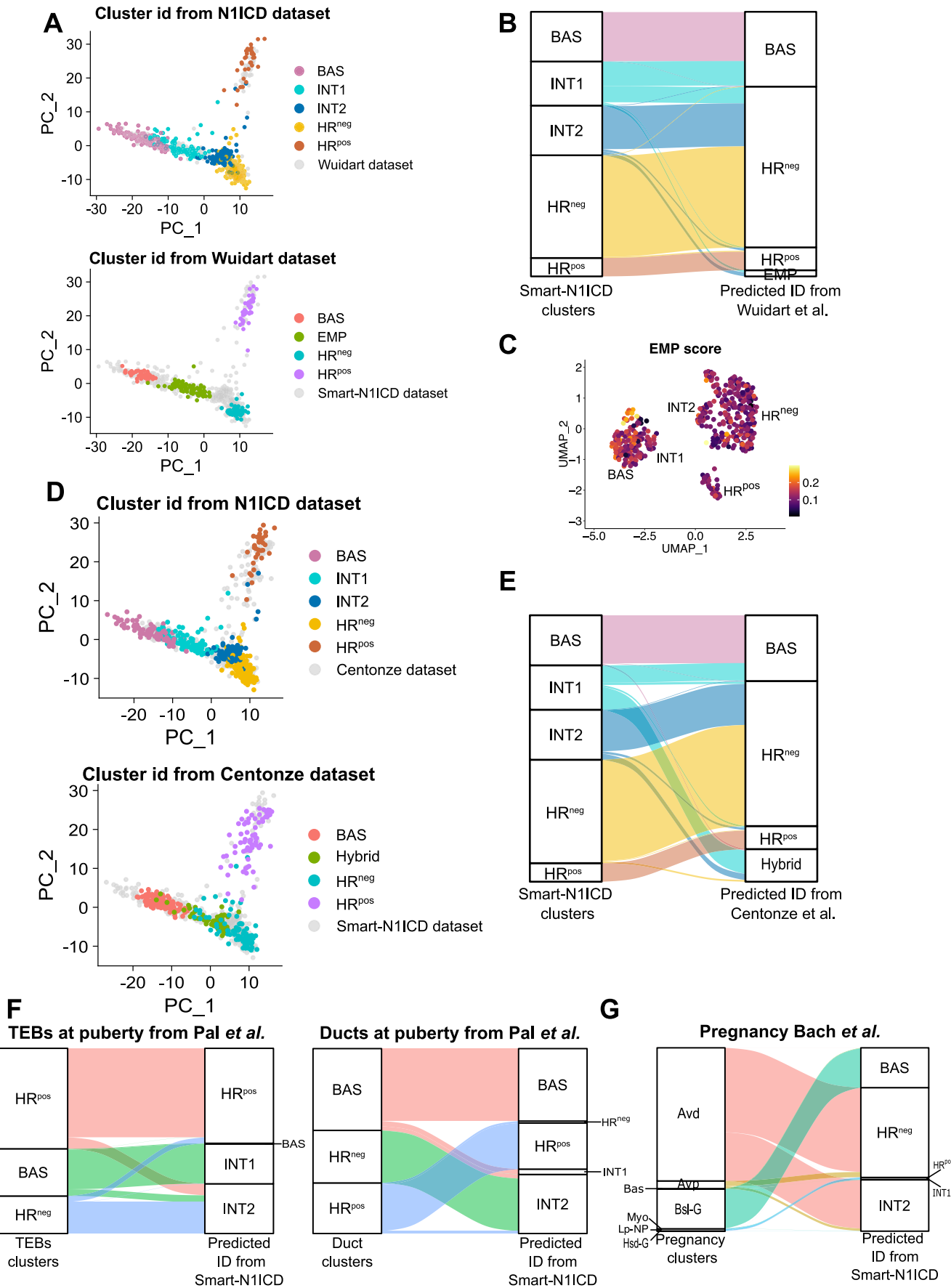
Related to Fig. 2. (A) Representative images of SMACre^{ERT2}/mTmG mammary gland (MG), lacrimal gland (LG), salivary gland (SG), and K5Cre^{ERT2}/mTmG prostate sections induced at P21 and analyzed 6 weeks later by immunofluorescence for mGFP (yellow) and the basal marker α -SMA (white), demonstrating that α -SMA^{pos} cells are exclusively BCs, indicating unipotency. Scale bars represent 50 μ m. (B) Gating strategy used for cell sorting experiments. Doublets, Lin^{pos} and dead cells (DAPI^{pos}) were excluded from further analysis. (C) FACS plots showing the gates defining luminal (EpCAM^{high}/Cd49^{low}), intermediate (EpCAM^{med}/Cd49^{fmed}), and basal (EpCAM^{low}/Cd49^{fhigh}) sorted cells from SMACre^{ERT2}/N1ICD mammary glands induced at P21 and chased for 1, 3, or 6 weeks, that were used for SMARTseqV2. The percentages of each population are indicated for one representative experiment. (D) UMAP plot showing the distribution of each sequenced cell in the different clusters based on the Cre mouse used to target them (K5Cre^{ERT2} or SMACre^{ERT2}). (E) UMAP plots show the expression of well-defined markers for basal (*Krt5*, *Krt14*), luminal (*Krt8*, *Krt19*), and HR^{pos} (*Esr1*, *Pgr*) cells. (F) Violin plots representing basal and luminal scores, based on signatures from (Kendrick et al, 2008) for each cluster. (G) UMAP plot indicating the cell cycle phases for each sequenced cell. The boxed cells correspond to the proliferative population highlighted in Fig. 2E. (H) UMAP plots are color-coded according to the expression of the single-cell G2M and S score. (I) Heatmap showing the genes presenting a high expression in the proliferative group. (J) Proportion of cells in different cell cycle phases (G1, G2M, or S) in each cluster.





◀ **Figure EV2. Lineage trajectories of cell fate switch and associated transcriptional features.**

Related to Fig. 3. (A) PCA plots highlighting changes in cell transcriptional state along the basal-luminal differentiation trajectory, at different timepoints following N1ICD activation. Colored dots indicate the FACS gate information for each index-sorted cell, based on the cell surface markers EpCAM and Cd49f. (B) UMAP refined analysis of the HR^{neg} cluster indicating GFP^{pos} and GFP^{neg} cells. The two new resulting subclusters appearing are indicated by different colors (turquoise and orange). The boxed graph represents the percentage of GFP^{pos} (green) and GFP^{neg} (gray) cells for each cluster, showing the predominance of GFP^{pos} mutant cells in the pre-luminal cluster. (C) UMAP representation of the distribution of mutant nGFP^{pos} cells belonging to the HR^{neg} cluster color-coded based on the different timepoints after N1ICD induction, as indicated. (D) UMAP representations of the two luminal subclusters show the expression levels of two luminal-specific genes (*Krt18* and *Epcam*) for each cell. (E) Heatmap showing differentially expressed genes (DEGs) distinguishing the pre-luminal and luminal clusters. (F) Volcano Plot of DEGs between HR^{neg} GFP^{pos} (170 cells) (red dots) and HR^{neg} GFP^{neg} (34 cells) (blue dots) cells. (G) Hematoxylin and Eosin staining of WT (wild-type) mammary gland and mammary tumor sections following N1ICD activation. Wild-type mouse mammary glands were collected after one pregnancy, while tumors were harvested after three pregnancies, followed by 10 to 15 days of involution. (H) Immunofluorescence anti-GFP (corresponding to N1ICD), α -SMA, and K8 on sections of mammary tumors developed upon N1ICD activation, three pregnancies, and 10 days of involution showing the clonal expansion of mutant nGFP^{pos} cells. Nuclei are stained with DAPI. Scale bars represent 100 μ m in (G, H).



◀ Figure EV3. Integration of SMART-N1ICD dataset with scRNAseq from embryonic and adult hybrid mammary cells.

Related to Fig. 3. (A) PCA plots showing the integration of our data (SMART-N1ICD) and the dataset from Wuidart et al (Wuidart et al, 2018). (B) Alluvium plots showing label transfer of each cell cluster using the Wuidart dataset as reference. (C) UMAP plot representing enrichment for the EMP score derived from Wuidart et al, dataset. Purple represents the lowest EMP score and yellow the highest EMPs score. (D) PCA plots showing the integration of our data (SMART-N1ICD) and the dataset from Centonze et al, (Centonze et al, 2020). (E) Alluvium plots showing label transfer of each cell cluster using the Centonze dataset as reference. (F) Alluvium plots showing label transfer of each cell cluster in the Pal et al, dataset either from TEBs (left) or from pubertal ducts (right) using our dataset (Smart-N1ICD) as reference. (G) Alluvium plot showing label transfer of each cell cluster in the Bach et al., dataset performed at pregnancy using our dataset (Smart-N1ICD) as reference.

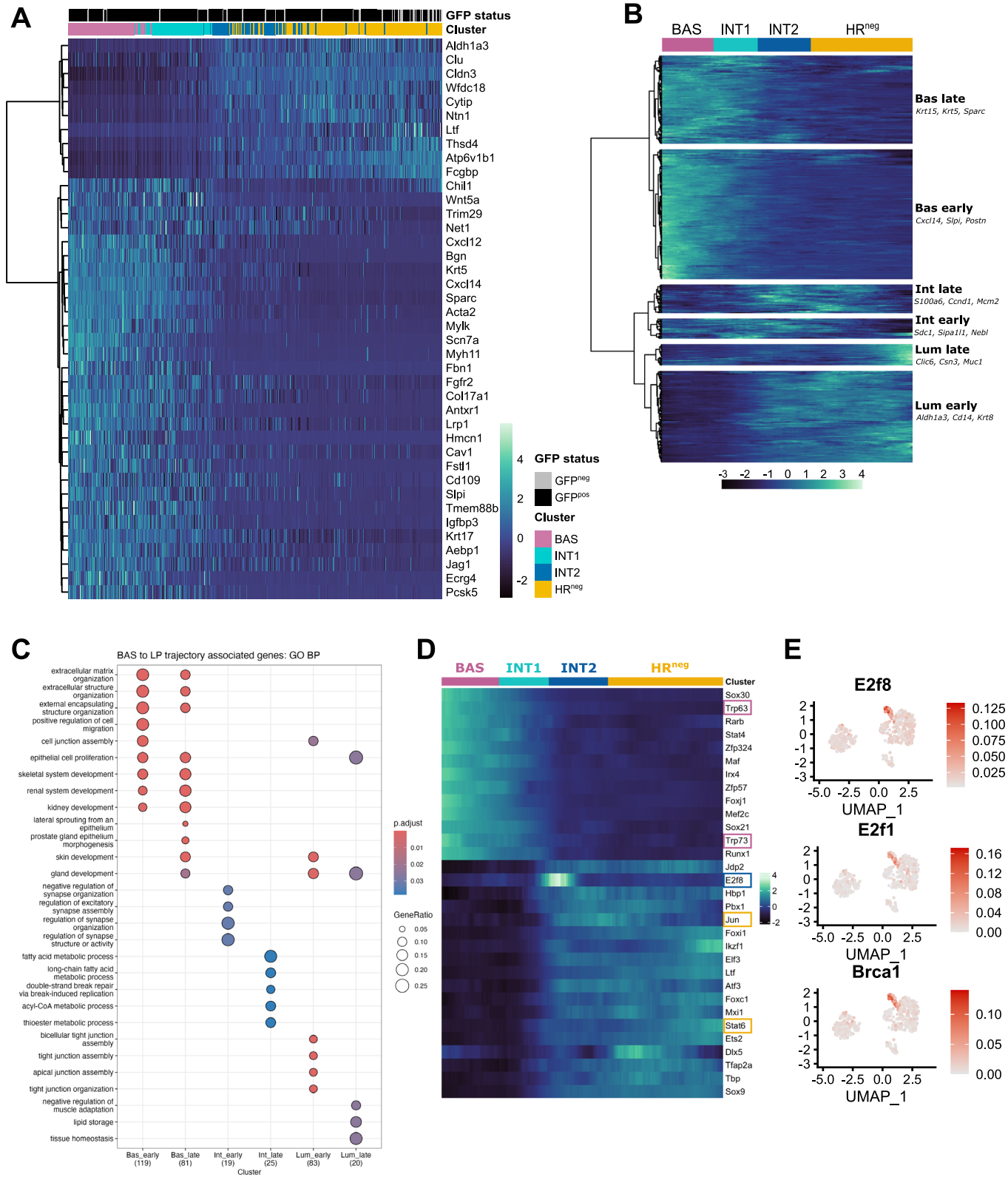


Figure EV4. Pseudotime ordering identifies the transcriptional signatures associated with the progressive lineage transition from BCs to LCs.

Related to Fig. 3. (A) Heatmap illustrating the top 40 genes exhibiting a differential pattern of expression along the pseudotime from basal to luminal (HR^{neg}) identity. The clusters are color-coded, as in Fig. 2B. (B) Heatmap showing the dynamic expression profile of each gene towards the lineage switch trajectory, distinguishing six different patterns of expression along the process of basal to luminal transition. (C) GO terms associated with the genes defining the six groups in (B). *p* values were defined using one-sided Fisher's exact statistical test. (D) Heatmap showing the top transcriptional regulons specific to each cluster (based on RSS analysis), plotted along the pseudotime trajectory 1. Boxed genes are described in the text. (E) UMAP plots showing the expression of regulons specific to cluster INT2, based on Fig. 3E.

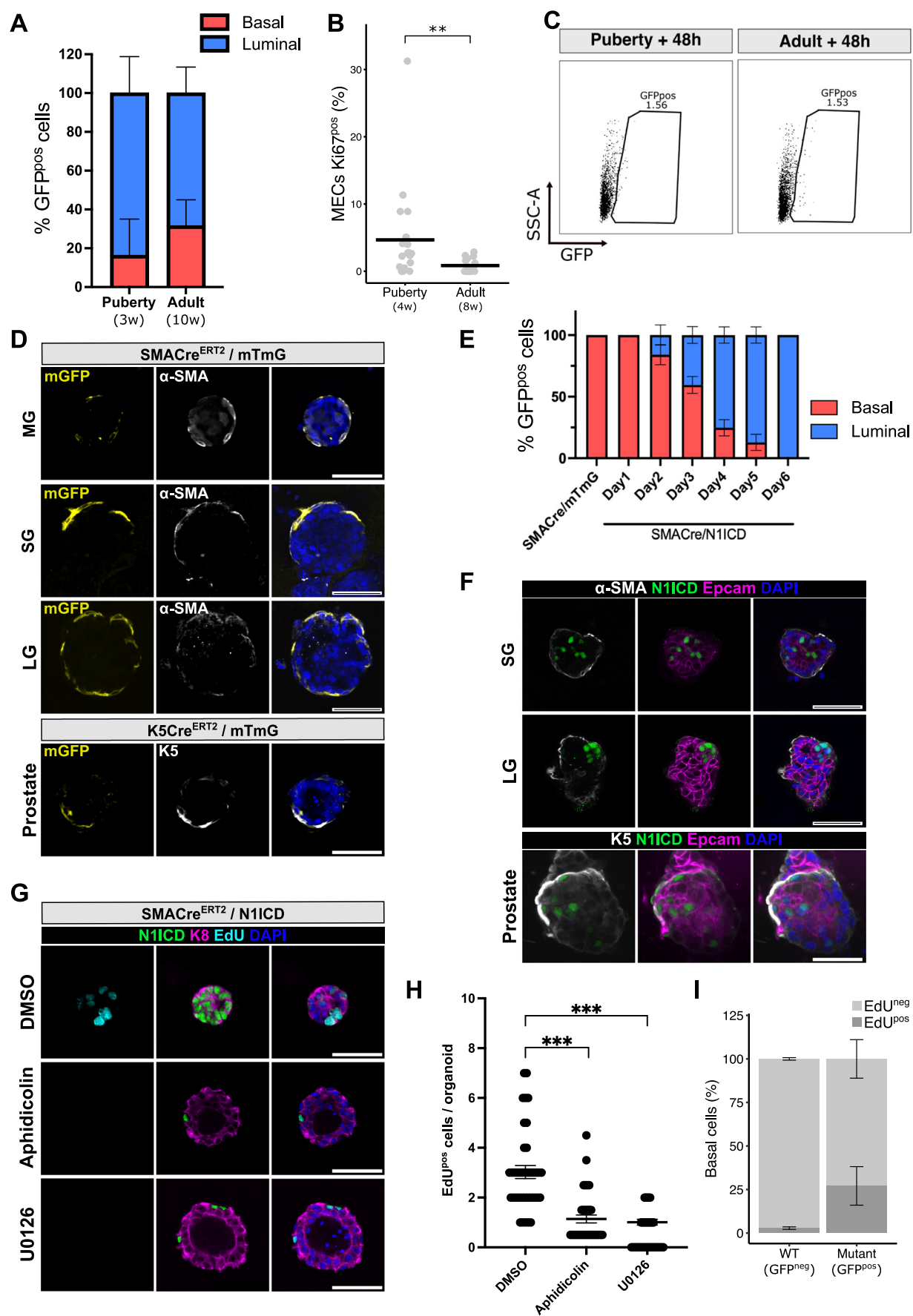
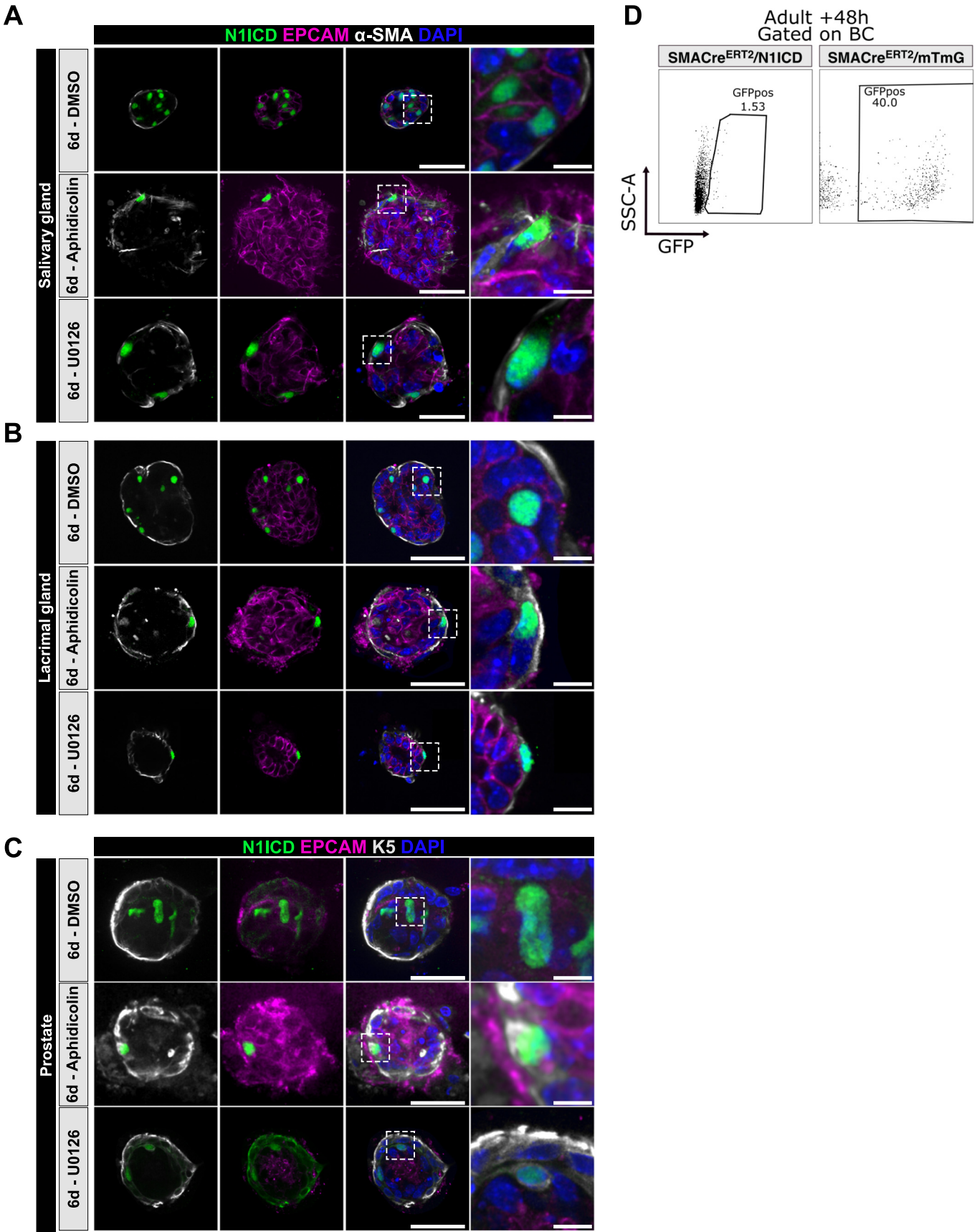


Figure EV5. Lineage switch and BCs unipotency are recapitulated in organoids.

Related to Figs. 4, 5. (A) Quantification of the proportion of GFP^{pos} cells in basal and luminal FACS gates after induction at pre-puberty (3w) or adulthood (10w), showing the incomplete cell fate switch after a 6-week chase in adult mice. Data were displayed as mean ± SEM, and represent at least three mice. (B) Percentage of Ki67-positive mammary epithelial cells (MECs) in pubertal (4w) or adult (8w) mice. $n = 3$ mice with a total of 16 sections for pubertal mice and 19 sections for adult mice. p value = 0.009 defined by t -test. (C) FACS plots showing the percentage of mutant GFP^{pos} cells after 48 h induction in pubertal or adult mice, demonstrating invariant recombination efficiency. (D) Representative immunofluorescence images showing mGFP, α -SMA, and K8 expression, 6 days after 4-OHT induction of SMACre^{ERT2}/mTmG control organoids. Scale bar 50 μ m. (E) Quantification of the proportion of mutant nGFP^{pos} basal and luminal cells per organoid at the indicated times after induction in SMACre^{ERT2}/N1ICD mice or 6 days after tamoxifen in SMACre^{ERT2}/mTmG control animals. Error bars represent mean ± SEM. A minimum of 18 organoids were analyzed for each condition and included three independent experiments. (F) Representative images of SMACre^{ERT2}/N1ICD (SG and LG) or K5Cre^{ERT2}/N1ICD (prostate) organoids showing mutant cells (nGFP^{pos}), featuring the expression of the luminal marker Epcam and the absence of basal markers (α -SMA or K5, as indicated). Scale bar 50 μ m. (G) Representative images of EdU staining in SMACre^{ERT2}/N1ICD organoids treated with DMSO, Aphidicolin, and U0126. Scale bar represents 50 μ m. (H) Quantification of EdU^{pos} cells per organoids after 6 days in culture with DMSO, Aphidicolin, or U0126. *** indicates p value <0.0001 (p value = 2.04e-10 for DMSO/Aphidicolin and 2.04e-10 for DMSO/U0126, using Wilcoxon test). Data were displayed as mean ± SEM and represented three independent experiments with at least 40 organoids in total. (I) Quantification of the proportion of EdU positive or negative WT (GFP^{neg}) or mutant (GFP^{pos}) BCs in organoids 3 days after induction. Data were displayed as mean ± SEM and 19 organoids were analyzed from three independent experiments.



◀ Figure EV6. Proliferation is an obligatory step for lineage transition in salivary, lacrimal, and prostate organoids.

Related to Fig. 4. (A–C) Representative images showing immunofluorescence for nGFP (N1ICD in green), the luminal marker Epcam (purple) and the basal gene α -SMA (white) in SMACre^{ERT2}/N1ICD salivary (A) and lacrimal (B) gland organoids, or the basal gene K5 (white) in K5Cre^{ERT2}/N1ICD prostate (C) organoids treated with DMSO, Aphidicolin or U0126 for 6 days. Nuclei are stained with DAPI in blue. The scale bar represents 50 μ m in (A–C) and 10 μ m in the magnified insets. (D) Quantification of the proportion of basal GFP^{pos} cells after 48 h of induction in SMACre^{ERT2}/N1ICD and SMACre^{ERT2}/mTmG adult mice, showing the difference in recombination efficiency with the two Cre drivers.