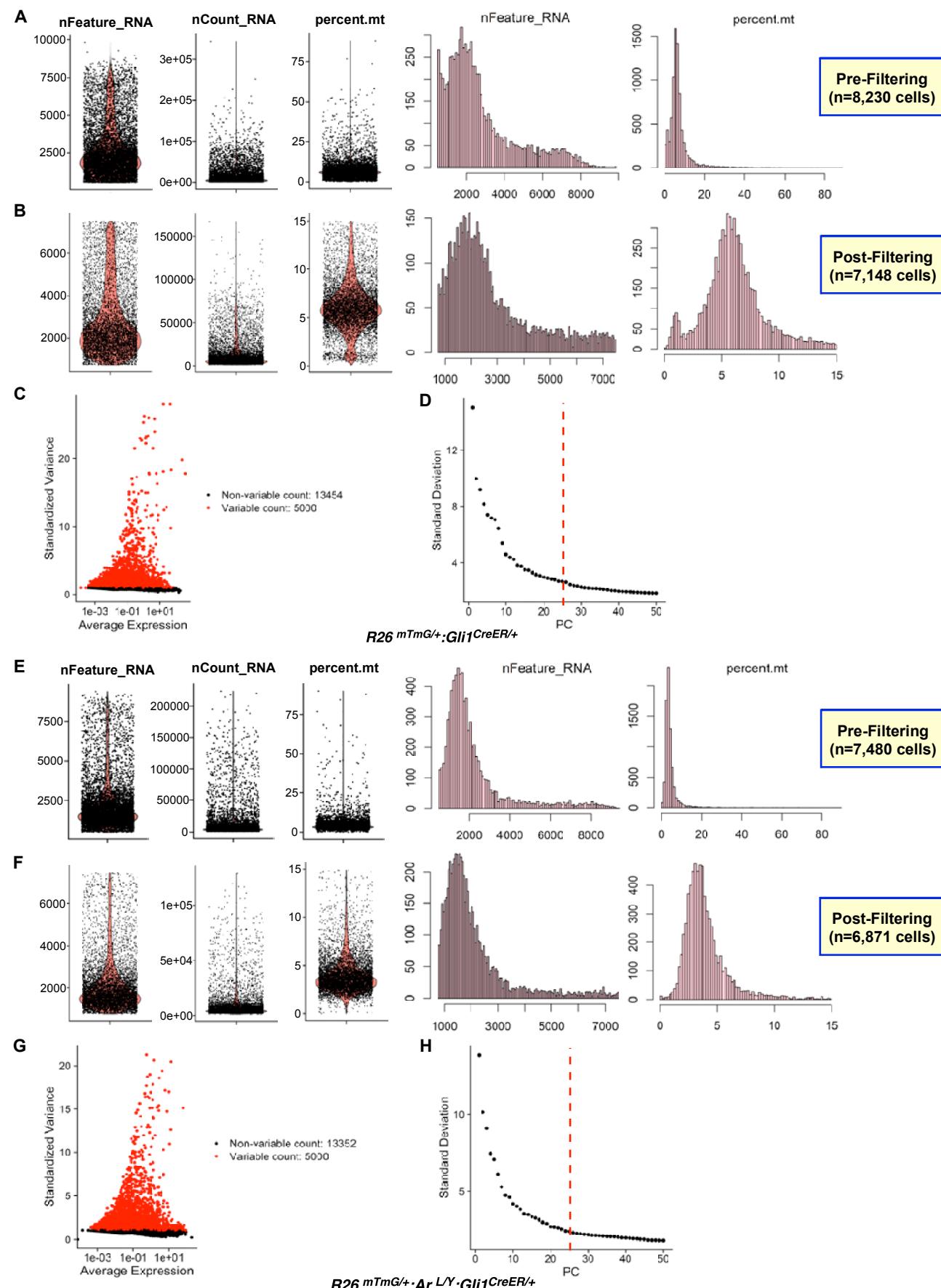
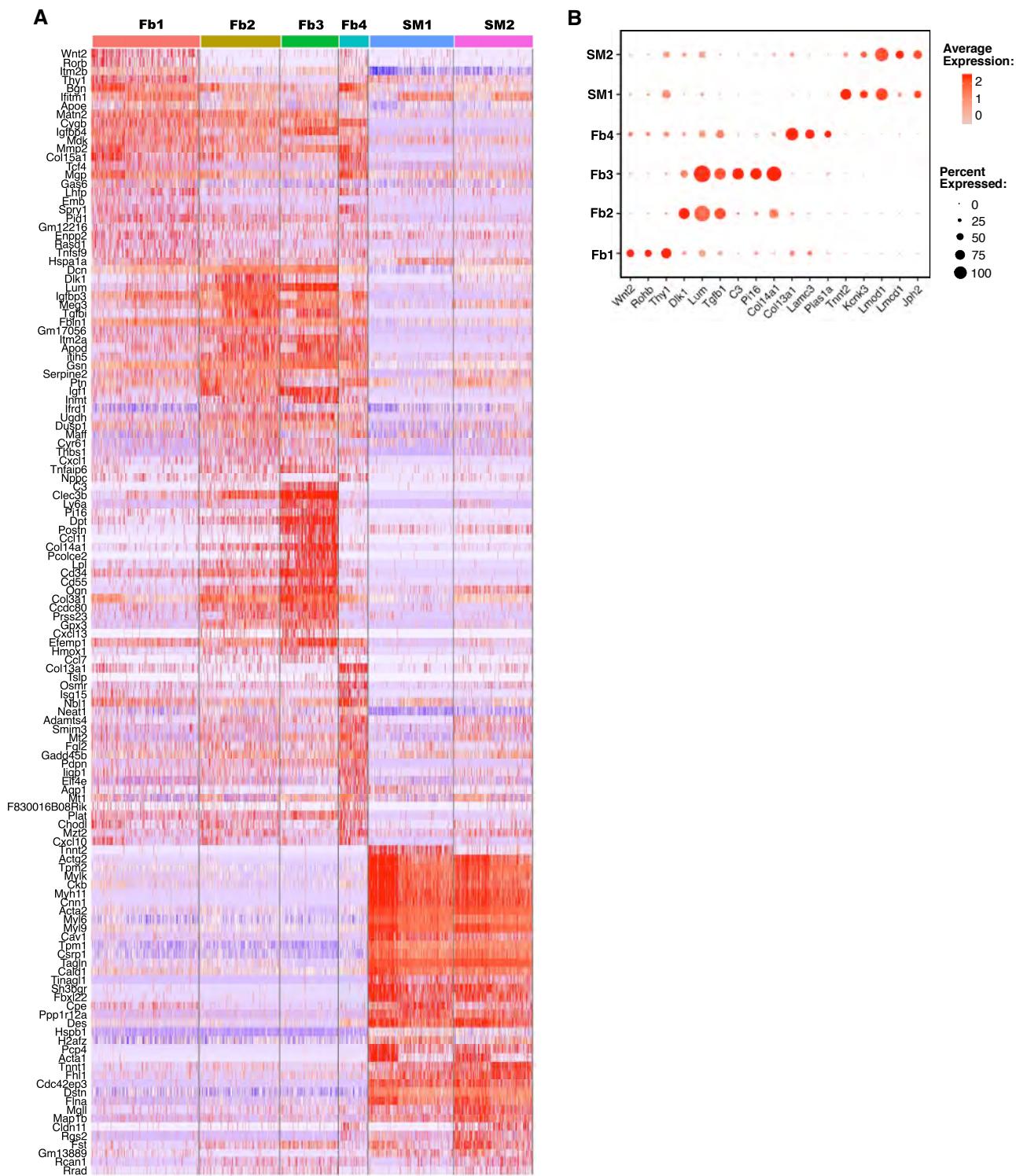


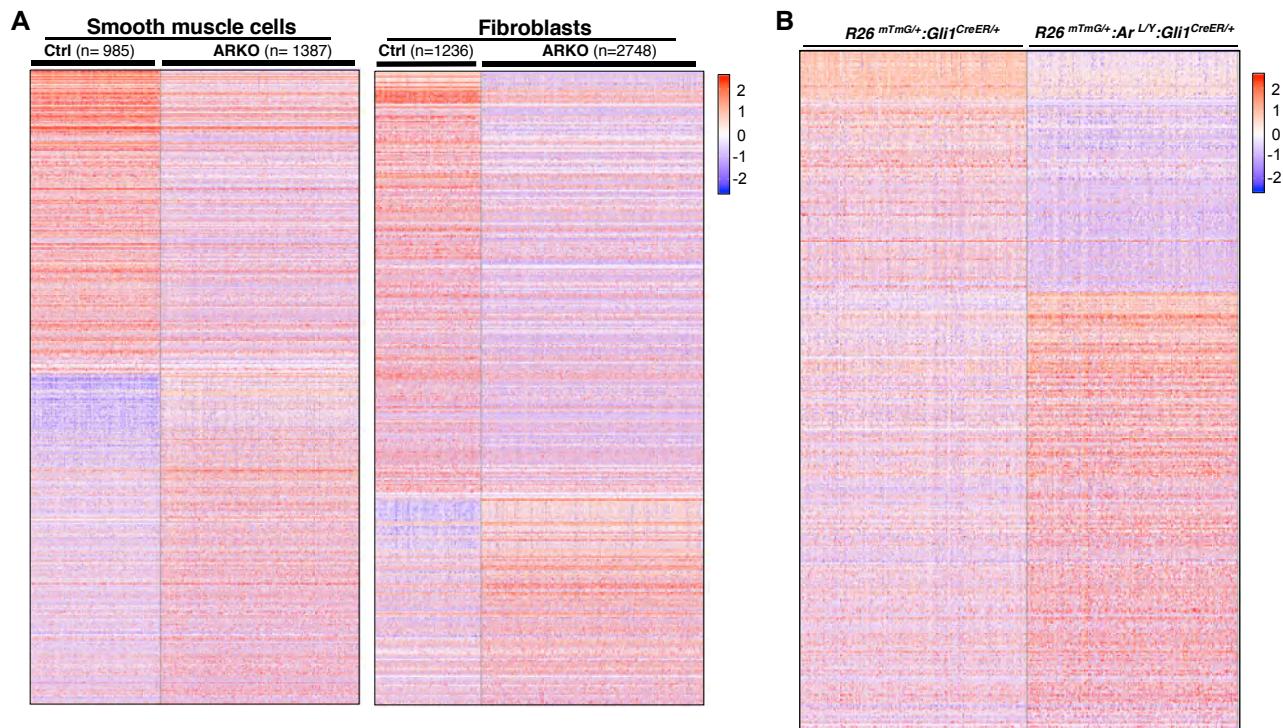
**Fig. S1. Gross and histological images of prostate tissues.** (A1-B2) Gross view images of P34 and P56 prostates collected from the indicated genotypes. (C) Ratio of prostate wet weight versus whole body weight as percentages (left) and serum testosterone concentrations (right) of mice of the indicated genotypes. Error bars indicate s.d.: \*\*P < 0.01; analyzed using 2-tailed t-test; n = 6 mice per genotype. Please also see detailed data on Supplemental Table S6.



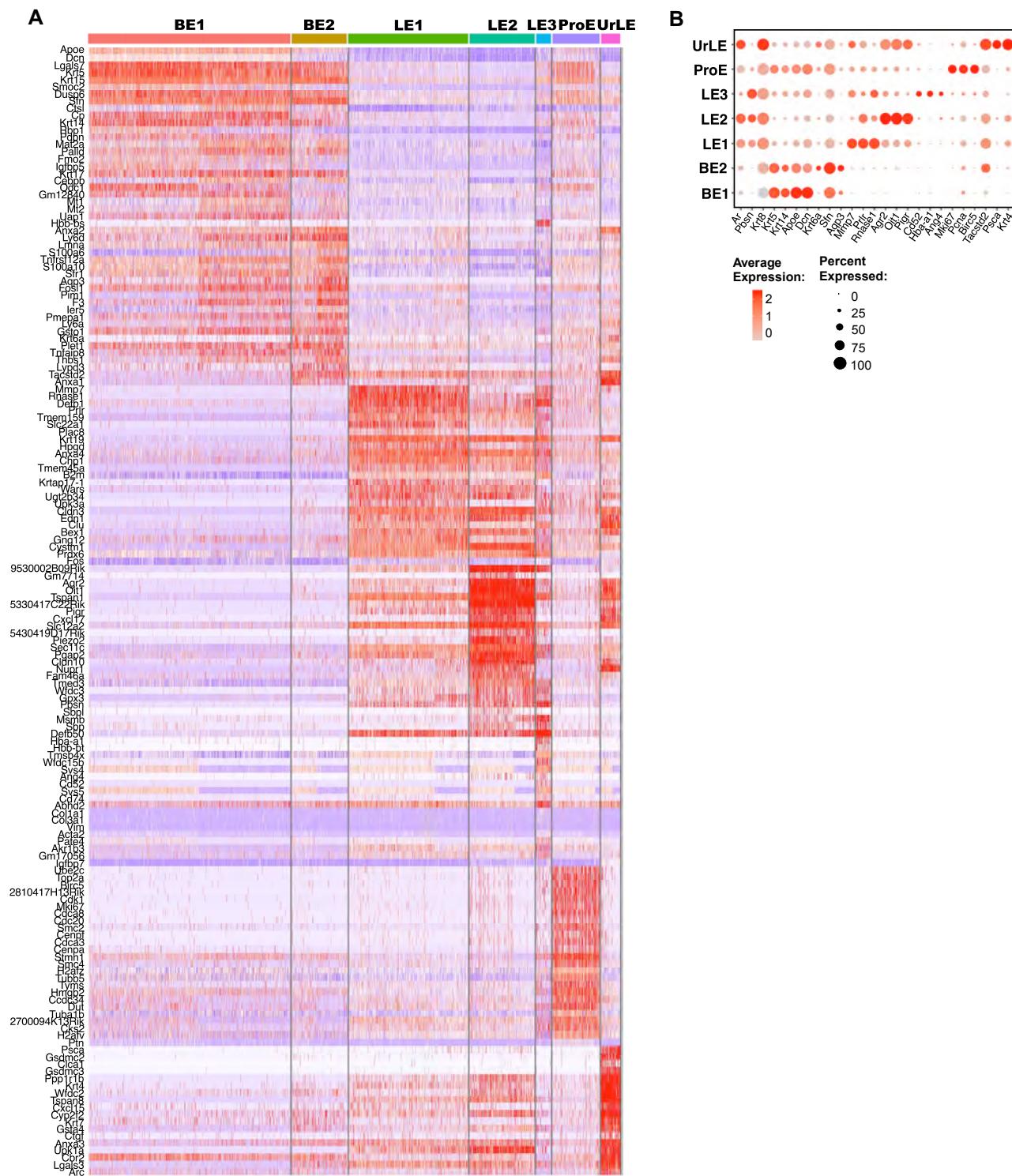
**Fig. S2. Quality controls and filtering of scRNAseq samples.** (A-B) Violin plots and histograms, visualizing the indicated metrics, pre- and post-filtering for the control sample. (C) Selection of variable genes used for principal component analysis of the control sample. (D) Elbow plot displaying cutoff for the number of PCs used for clustering the control sample. (E-H) Similar plots as described for A-D used for filtering the ARKO sample.



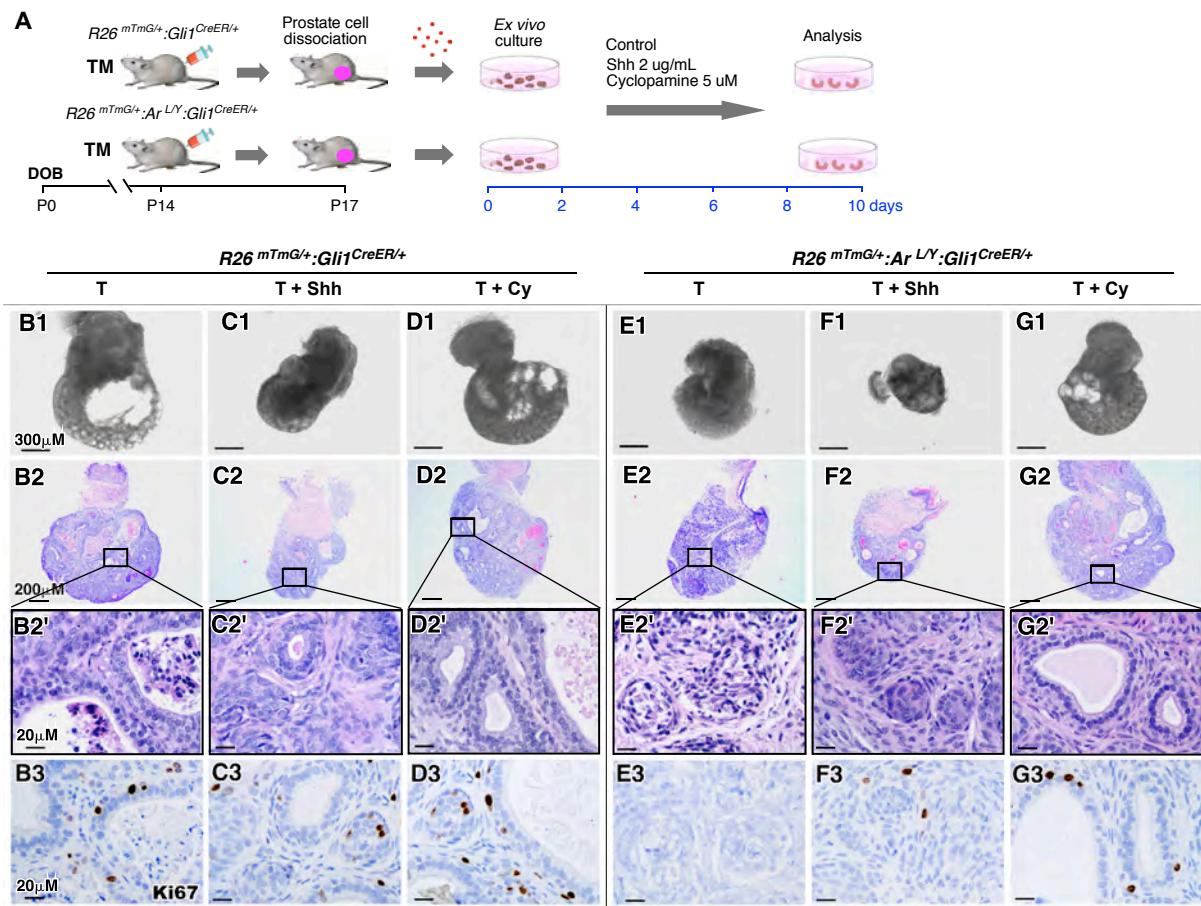
**Fig. S3. Transcriptomic profiles of prostate stromal cells.** (A) A heatmap was shown for DEGs in each of stromal cell clusters (please also see Figure 3A-D). (B) Dot plot of highly specific genes for each cellular cluster.



**Fig. S4. Heatmaps from major cell comparisons.** (A) Heatmaps displaying DEGs identified between the indicated samples in smooth muscle cells and fibroblasts. (B) A heatmap displaying DEGs between ARKO and control basal epithelial cells.



**Fig. S5. Transcriptomic profile analyses of prostate epithelial cells.** (A) A heatmap was shown for DEGs in each of prostatic epithelial cell clusters. (B) Dot plot of highly specific genes for each cellular cluster.

**Fig. S6.** The effect of Shh in prostatic epithelial morphogenesis and growth in organotypic cultures.

(A) Schematic of experiment, including knock out of AR in Gli1-positive cells, prostate collection, ex-vivo culture in the presence of Shh or cyclopamine, and analysis. (B1-D1 and E1-G1) Whole mount ventral prostate organs were maintained after 10 days in 10-9 M testosterone (B1 and E1), in the presence of 2  $\mu$ g/mL Shh (C1 and F1), or 5  $\mu$ M cyclopamine (D1 and G1). (B3-G3) H&E or Ki67 IHC staining from the cultured tissues with the indicated genotypes. T, testosterone; Cy, Cyclopamine.

**Table S1.** Fibroblast & SMC subcluster DEG List

[Click here to download Table S1](#)

**Table S2.** Bulk RNA-Seq generated differentially expressed genes between GFP+ ARKO and WT cells at P34

[Click here to download Table S2](#)

**Table S3.** GFP+ Fibroblast Control vs. ARKO DEG List

[Click here to download Table S3](#)

**Table S4.** GFP+ Smooth Muscle, Control vs. ARKO DEG List

[Click here to download Table S4](#)

**Table S5.** Basal Cells, Control vs. ARKO DEG List

[Click here to download Table S5](#)

**Table S6.** Percentage of Prostate weight per Body weight and mouse serum testosterone levels for Figure 1C.

| P35      |                    |            |              |          |              |                      |
|----------|--------------------|------------|--------------|----------|--------------|----------------------|
| Mouse ID | Genotype           | Age (days) | Prostate (g) | Body (g) | % Pro/Body   | Testosterone (ng/mL) |
| A0310    | mTmGL/+ Gli1CreER+ | 36         | 0.022        | 22.1     | 0.100        |                      |
| A0316    | mTmGL/+ Gli1CreER+ | 35         | 0.025        | 24.1     | 0.104        | 0.457                |
| A0197    | mTmGL/+ Gli1CreER+ | 34         | 0.015        | 25.6     | 0.059        | 0.587                |
| s0710    | mTmGL/+ Gli1CreER+ | 34         | 0.01         | 18.5     | 0.054        | 0.305                |
| A0417    | mTmGL/+ Gli1CreER+ | 36         | 0.019        | 23.9     | 0.079        |                      |
| A0418    | mTmGL/+ Gli1CreER+ | 35         | 0.018        | 25.5     | 0.071        | 0.287                |
| AVERAGE  |                    |            |              |          | <b>0.078</b> | <b>0.409</b>         |
| S.D      |                    |            |              |          | <b>0.019</b> | <b>0.122</b>         |

| Mouse ID | Genotype                 | Age (days) | Prostate (g) | Body (g) | % Pro/Body   | Testosterone (ng/mL) |
|----------|--------------------------|------------|--------------|----------|--------------|----------------------|
| A0318    | ArL/Y mTmGL/+ Gli1CreER+ | 34         | 0.003        | 21.7     | 0.014        | 0.412                |
| A0218    | ArL/Y mTmGL/+ Gli1CreER+ | 34         | 0.007        | 23.4     | 0.030        | 0.286                |
| s0711    | ArL/Y mTmGL/+ Gli1CreER+ | 35         | 0.005        | 24.1     | 0.021        | 0.345                |
| s0713    | ArL/Y mTmGL/+ Gli1CreER+ | 35         | 0.001        | 24.8     | 0.004        | 0.334                |
| s0706    | ArL/Y mTmGL/+ Gli1CreER+ | 36         | 0.002        | 25.1     | 0.008        | 0.297                |
| s0707    | ArL/Y mTmGL/+ Gli1CreER+ | 34         | 0.004        | 24.3     | 0.016        | 0.405                |
| AVERAGE  |                          |            |              |          | <b>0.015</b> | <b>0.347</b>         |
| S.D      |                          |            |              |          | <b>0.009</b> | <b>0.048</b>         |

| P56      |                    |            |              |          |              |                      |
|----------|--------------------|------------|--------------|----------|--------------|----------------------|
| Mouse ID | Genotype           | Age (days) | Prostate (g) | Body (g) | % Pro/Body   | Testosterone (ng/mL) |
| s6099    | mTmGL/+ Gli1CreER+ | 56         | 0.066        | 30.6     | 0.216        | 0.931                |
| s6175    | mTmGL/+ Gli1CreER+ | 56         | 0.056        | 28.5     | 0.196        | 0.866                |
| s6176    | mTmGL/+ Gli1CreER+ | 55         | 0.078        | 26.8     | 0.291        | 0.501                |
| s0410    | mTmGL/+ Gli1CreER+ | 60         | 0.048        | 28.9     | 0.166        | 0.61                 |
| s7344    | mTmGL/+ Gli1CreER+ | 57         | 0.053        | 26.3     | 0.202        | 0.712                |
| s7457    | mTmGL/+ Gli1CreER+ | 58         | 0.072        | 25.8     | 0.279        | 0.735                |
| AVERAGE  |                    |            |              |          | <b>0.225</b> | <b>0.726</b>         |
| S.D      |                    |            |              |          | <b>0.045</b> | <b>0.145</b>         |

| Mouse ID | Genotype                 | Age (days) | Prostate (g) | Body (g) | % Pro/Body   | Testosterone (ng/mL) |
|----------|--------------------------|------------|--------------|----------|--------------|----------------------|
| s5910    | ArL/Y mTmGL/+ Gli1CreER+ | 62         | 0.008        | 28.4     | 0.028        | 0.821                |
| s6083    | ArL/Y mTmGL/+ Gli1CreER+ | 60         | 0.018        | 31.1     | 0.058        | 0.609                |
| s6086    | ArL/Y mTmGL/+ Gli1CreER+ | 53         | 0.021        | 30.2     | 0.070        | 0.627                |
| s6360    | ArL/Y mTmGL/+ Gli1CreER+ | 58         | 0.017        | 33.8     | 0.050        | 0.478                |
| s0411    | ArL/Y mTmGL/+ Gli1CreER+ | 59         | 0.007        | 23.2     | 0.030        | 0.531                |
| s8610    | ArL/Y mTmGL/+ Gli1CreER+ | 55         | 0.017        | 31.4     | 0.054        | 0.788                |
| AVERAGE  |                          |            |              |          | <b>0.048</b> | <b>0.642</b>         |
| S.D      |                          |            |              |          | <b>0.015</b> | <b>0.125</b>         |

**Table S7.** Overall cell distribution and Cell counts. Supporting data for Figure 2H.

| Cell Type    | Cell Counts |             | Cell Distributions |                |
|--------------|-------------|-------------|--------------------|----------------|
|              | WT          | ARKO        | WT                 | ARKO           |
| Basal        | 1496        | 1396        | 20.93%             | 20.32%         |
| Lum          | 1644        | 633         | 23.00%             | 9.21%          |
| Pro Epi      | 484         | 47          | 6.77%              | 0.68%          |
| SV           | 404         | 56          | 5.65%              | 0.82%          |
| Club         | 89          | 66          | 1.25%              | 0.96%          |
| OE           | 93          | 26          | 1.30%              | 0.38%          |
| Fib          | 1239        | 2749        | 17.33%             | 40.01%         |
| SMC          | 982         | 1388        | 13.74%             | 20.20%         |
| Pro Stro     | 146         | 9           | 2.04%              | 0.13%          |
| Leuk         | 168         | 143         | 2.35%              | 2.08%          |
| Lymph        | 214         | 161         | 2.99%              | 2.34%          |
| Endo         | 117         | 70          | 1.64%              | 1.02%          |
| Peri         | 44          | 96          | 0.62%              | 1.40%          |
| MyoE         | 28          | 31          | 0.39%              | 0.45%          |
| <b>Total</b> | <b>7148</b> | <b>6871</b> | <b>100.00%</b>     | <b>100.00%</b> |

**Table S8.** Analysis of Fibroblast and Smooth Muscle cells. Supporting data for Figure 3. Supporting data for Figure 3E.

| Cluster      | Cell Counts |             | Cell Distributions |                |
|--------------|-------------|-------------|--------------------|----------------|
|              | WT          | ARKO        | WT                 | ARKO           |
| Fb1          | 486         | 1083        | 21.88%             | 26.19%         |
| Fb2          | 313         | 846         | 14.09%             | 20.46%         |
| Fb3          | 240         | 594         | 10.81%             | 14.37%         |
| Fb4          | 197         | 225         | 8.87%              | 5.44%          |
| SM1          | 435         | 799         | 19.59%             | 19.32%         |
| SM2          | 550         | 588         | 24.76%             | 14.22%         |
| <b>Total</b> | <b>2221</b> | <b>4135</b> | <b>100.00%</b>     | <b>100.00%</b> |

**Supporting data for Figure 3H.**

| Gli1 & Ar Exp |           | Cells | Percentages |
|---------------|-----------|-------|-------------|
| ARKO          | Gli1+ Ar+ | 107   | 2.59%       |
|               | Gli1+ Ar- | 271   | 6.55%       |
| WT            | Gli1+ Ar+ | 84    | 3.78%       |
|               | Gli1+ Ar- | 64    | 2.88%       |

| Total Cell Counts |           | Fb1 | Fb2 | Fb3 | Fb4 | SM1 | SM2 | Total | Percentages |
|-------------------|-----------|-----|-----|-----|-----|-----|-----|-------|-------------|
| ARKO              | Gli1+ Ar+ | 20  | 27  | 30  | 8   | 4   | 18  | 107   | 2.59%       |
|                   | Gli1+ Ar- | 70  | 69  | 43  | 13  | 32  | 44  | 271   | 6.55%       |
|                   | Gli1- Ar+ | 245 | 226 | 210 | 62  | 192 | 156 | 1091  | 26.38%      |
|                   | Gli1- Ar- | 748 | 524 | 311 | 142 | 571 | 370 | 2666  | 64.47%      |
| WT                | Gli1+ Ar+ | 11  | 14  | 22  | 6   | 13  | 18  | 84    | 3.78%       |
|                   | Gli1+ Ar- | 19  | 10  | 11  | 7   | 5   | 12  | 64    | 2.88%       |
|                   | Gli1- Ar+ | 206 | 161 | 133 | 103 | 211 | 359 | 1173  | 52.81%      |
|                   | Gli1- Ar- | 250 | 128 | 74  | 81  | 206 | 161 | 900   | 40.52%      |

**Supporting data for Figure 3I.**

| Comparison                        | Name                                   | NES     | -log10(FDR) |
|-----------------------------------|--|---------|-------------|
| Gli1+Ar+ FB<br>VS.<br>Gli1+Ar- FB | GO_PROSTATE_GLAND_MORPHOGENESIS        | 2.24080 | 1.23938881  |
|                                   | GO_RESPONSE_TO_TESTOSTERONE            | 2.09845 | 1.00767115  |
|                                   | GO_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY | 2.08330 | 1.00248619  |
|                                   | KEGG_PROSTATE_CANCER                   | 2.06041 | 1.04735116  |
| Gli1+Ar+ FB<br>VS.<br>Total FB    | GO_RESPONSE_TO_TESTOSTERONE            | 2.10031 | 1.22787940  |
|                                   | GO_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY | 2.08591 | 1.20424383  |
|                                   | GO_PROSTATE_GLAND_MORPHOGENESIS        | 1.89648 | 1.07953058  |
|                                   | KEGG_PROSTATE_CANCER                   | 1.85064 | 1.04298875  |
| Gli1+Ar+ SM<br>VS.<br>Gli1+Ar- SM | GO_PROSTATE_GLAND_MORPHOGENESIS        | 2.36983 | 2.30152146  |
|                                   | KEGG_PROSTATE_CANCER                   | 2.19818 | 1.62870485  |
|                                   | GO_RESPONSE_TO_TESTOSTERONE            | 2.16666 | 1.59189492  |
|                                   | GO_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY | 1.89275 | 1.37063287  |
| Gli1+Ar+ SM<br>VS.<br>Total SM    | GO_PROSTATE_GLAND_MORPHOGENESIS        | 2.33231 | 2.74811927  |
|                                   | KEGG_PROSTATE_CANCER                   | 2.15565 | 2.10510871  |
|                                   | GO_RESPONSE_TO_TESTOSTERONE            | 2.07556 | 1.89213969  |
|                                   | GO_ANDROGEN_RECEPTOR_SIGNALING_PATHWAY | 1.83736 | 1.40657923  |

**Table S9.** Analysis of mGFP+ Fibroblast and Smooth Muscle cells. Supporting data for Figure 4.

Support data for Figure 4D. qPCR of Shh axes genes.

| Genes | cDNA amount | Sample Label | Ct1    | Ct2    | Ct3    | Fold Change 1 | Fold Change 2 | Fold Change 3 | Average | SD    |
|-------|-------------|--------------|--------|--------|--------|---------------|---------------|---------------|---------|-------|
| AR    | 15 ng       | P34-WT       | 28.005 | 28.105 | 27.995 | 0.338         | 0.394         | 0.279         | 0.337   | 0.058 |
|       | 15 ng       | P34-ARKO     | 29.742 | 29.664 | 30.042 |               |               |               |         |       |
| Gli1  | 15 ng       | P34-WT       | 38.375 | 38.375 | 38.375 | 9.617         | 11.128        | 9.967         | 10.237  | 0.791 |
|       | 15 ng       | P34-ARKO     | 34.913 | 34.791 | 34.891 |               |               |               |         |       |
| Ptc1  | 15 ng       | P34-WT       | 36.176 | 35.214 | 35.224 | 2.359         | 2.879         | 3.120         | 2.786   | 0.389 |
|       | 15 ng       | P34-ARKO     | 34.642 | 33.581 | 33.482 |               |               |               |         |       |
| Smo   | 15 ng       | P34-WT       | 31.869 | 31.777 | 31.797 | 0.997         | 1.448         | 1.293         | 1.223   | 0.319 |
|       | 15 ng       | P34-ARKO     | 31.577 | 31.036 | 31.324 |               |               |               |         |       |

Support data for Figure 4E. GSEA pathways of mGFP+ Smooth Muscle Cells

| mGFP+ Smooth Muscle ARKO vs WT GSEA  |          |             |  |
|--|----------|-------------|--|
| NAME   | NES      | -LOG10(FDR) |  |
| HALLMARK_P53_PATHWAY   | 2.11784  | 3.729001813 |  |
| HALLMARK_HYPOXIA   | 1.963032 | 2.406787858 |  |
| INGRAM_SHH_TARGETS_UP  | 1.763542 | 1.47393823  |  |
| GLI1_UP_V1_UP  | 1.65949  | 1.207757537 |  |
| GO_REGULATION_OF_CELLULAR_RESPONSE_TO_TRANSFORMING_GROWTH_FACTOR_BETA_STIMULUS | 1.660446 | 1.080226077 |  |
| GO_NEGATIVE_REGULATION_OF_CELL_GROWTH  | 1.645277 | 1.063061118 |  |
| HALLMARK_WNT_BETA_CATENIN_SIGNALING  | 1.478983 | 0.764735821 |  |
| HALLMARK_TGF_BETA_SIGNALING  | 1.416184 | 0.668037768 |  |
| GO_POSITIVE_REGULATION_OF_CELL_CYCLE_ARREST                                    | 1.371023 | 0.612146438 |  |
| GO_REGULATION_OF_FIBROBLAST_MIGRATION  | 1.365376 | 0.602691428 |  |
| KEGG_OXIDATIVE_PHOSPHORYLATION   | -2.83683 | 4           |  |
| GO_ATP_SYNTHESIS_COUPLED_ELECTRON_TRANSPORT                                    | -2.7257  | 4           |  |
| GO_MYOFIBRIL_ASSEMBLY  | -2.1484  | 2.912193959 |  |
| GO_MESENCHYME_MORPHOGENESIS  | -2.08063 | 2.347190766 |  |
| KEGG_CALCIUM_SIGNALING_PATHWAY   | -2.04117 | 2.132914742 |  |
| KEGG_ECM_RECECTOR_INTERACTION  | -1.97932 | 1.897432905 |  |
| GO_COLLAGEN_FIBRIL_ORGANIZATION  | -1.97805 | 1.895303269 |  |
| GO_TISSUE_MORPHOGENESIS  | -1.94423 | 1.77504176  |  |
| GO_SMOOTH_MUSCLE_CONTRACTION   | -1.77207 | 1.188126626 |  |
| GO_INNATE_IMMUNE_RESPONSE_ACTIVATING_CELL_SURFACE_RECECTOR_SIGNALING_PATHWAY   | -1.76945 | 1.182395665 |  |

Support data for Figure 4F. GSEA results of mGFP+ Fibroblast Cells

| mGFP+ Fibroblasts ARKO vs WT GSEA  |            |             |  |
|--|------------|-------------|--|
| NAME   | NES        | -LOG10(FDR) |  |
| KEGG_ECM_RECECTOR_INTERACTION  | -2.235573  | 4           |  |
| KEGG_OXIDATIVE_PHOSPHORYLATION   | -2.234102  | 4           |  |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION                                   | -2.0828555 | 4           |  |
| KEGG_FOCAL_ADHESION  | -1.9791621 | 3.165389278 |  |
| GO_INNATE_IMMUNE_RESPONSE_ACTIVATING_CELL_SURFACE_RECECTOR_SIGNALING_PATHWAY | -1.9208753 | 2.617685414 |  |
| GO_BASEMENT_MEMBRANE_ORGANIZATION  | -1.8303815 | 1.994084941 |  |
| GO_REGULATION_OF_STEM_CELL_DIFFERENTIATION                                   | -1.6908146 | 1.277677598 |  |
| GO_ORGAN_GROWTH  | -1.6186373 | 0.95427437  |  |
| GO_POSITIVE_REGULATION_OF_CANONICAL_WNT_SIGNALING_PATHWAY                    | -1.6166953 | 0.947127453 |  |
| HALLMARK_INTERFERON_ALPHA_RESPONSE   | -1.5846622 | 0.858965717 |  |
| KEGG_RIBOSOME  | 3.2092416  | 4           |  |
| GO_TRANSLATIONAL_INITIATION  | 2.8199763  | 4           |  |
| GO_RNA_CATABOLIC_PROCESS   | 2.2612283  | 3.070149549 |  |
| GO_CYTOPLASMIC_PATTERN_RECOGNITION_RECECTOR_SIGNALING_PATHWAY                | 1.9987289  | 1.432116977 |  |
| HALLMARK_TNFA_SIGNALING_VIA_NFKB   | 1.9932461  | 1.479435865 |  |
| GO_CELLULAR_RESPONSE_TO_CALCIUM_ION  | 1.9727091  | 1.411573656 |  |
| HALLMARK_HYPOXIA   | 1.8178613  | 0.979670752 |  |
| INGRAM_SHH_TARGETS_UP  | 1.7212644  | 1.01322108  |  |
| GO_NEGATIVE_REGULATION_OF_SMOOTH_MUSCLE_CELL_PROLIFERATION                   | 1.6806989  | 0.715143776 |  |
| GO_REGULATION_OF_NON_CANONICAL_WNT_SIGNALING_PATHWAY                         | 1.6569313  | 0.679290515 |  |

**Table S10.** Analysis and GSEA pathways of Epithelial cell. Supporting data for Figure 5. **Support data**

for Figure 5E. Epithelial Cell Counts and Distributions

| Cluster       | Cell Counts |             | Cell Distributions |             |
|---------------|-------------|-------------|--------------------|-------------|
|               | WT          | ARKO        | WT                 | ARKO        |
| Basal1        | 1211        | 1033        | 32.82%             | 48.54%      |
| Basal2        | 262         | 351         | 7.10%              | 16.49%      |
| Luminal1      | 958         | 376         | 25.96%             | 17.67%      |
| Luminal2      | 490         | 234         | 13.28%             | 11.00%      |
| Luminal3      | 161         | 3           | 4.36%              | 0.14%       |
| Proliferating | 487         | 47          | 13.20%             | 2.21%       |
| UrL           | 121         | 84          | 3.28%              | 3.95%       |
| Total         | <b>3690</b> | <b>2128</b> | <b>100%</b>        | <b>100%</b> |

**Support data for Figure 5G.** GSEA pathways from Basal Epithelial Cells

| Basal Cells ARKO vs WT GSEA                                   |  | NES      | -Log10(FDR) |
|---|--|----------|-------------|
| NAME  |  |          |             |
| HALLMARK_TNFA_SIGNALING_VIA_NFKB                              |  | 2.311912 | 4           |
| HALLMARK_HYPOXIA  |  | 2.164371 | 4           |
| GO_TRANSFORMING_GROWTH_FACTOR_BETA_RECEPTOR_SIGNALING_PATHWAY |  | 2.107666 | 3.107348966 |
| GO_CELL_CELL_JUNCTION_ORGANIZATION                            |  | 1.98715  | 2.605339453 |
| HALLMARK_ESTROGEN_RESPONSE_EARLY                              |  | 1.923271 | 2.225282794 |
| HALLMARK_IL6_JAK_STAT3_SIGNALING                              |  | 1.908292 | 2.141488909 |
| GO_CELLULAR_RESPONSE_TO_GLUCOSE_STARVATION                    |  | 1.726781 | 1.471250792 |
| GO_POSITIVE_REGULATION_OF_NOTCH_SIGNALING_PATHWAY             |  | 1.720294 | 1.450930004 |
| HALLMARK_HEDGEHOG_SIGNALING                                   |  | 1.423183 | 0.837334379 |
| KEGG_WNT_SIGNALING_PATHWAY                                    |  | 1.274603 | 0.607475445 |
| KEGG_OXIDATIVE_PHOSPHORYLATION                                |  | -3.04033 | 4           |
| GO_CELLULAR_RESPIRATION                                       |  | -2.79106 | 4           |
| KEGG_CITRATE_CYCLE_TCA_CYCLE                                  |  | -2.43539 | 4           |
| GO_GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY             |  | -2.42321 | 4           |
| HALLMARK_INTERFERON_ALPHA_RESPONSE                            |  | -1.71164 | 1.345958293 |
| GO_REGULATION_OF_MORPHOGENESIS_OF_AN_EPITHELIUM               |  | -1.69853 | 1.321810753 |
| HALLMARK_MYC_TARGETS_V1                                       |  | -1.64103 | 1.172525397 |
| HALLMARK_ANGIOGENESIS   |  | -1.59576 | 1.079492869 |
| HALLMARK_GLYCOLYSIS   |  | -1.53481 | 0.935535773 |
| GO_MORPHOGENESIS_OF_A_POLARIZED_EPITHELIUM                    |  | -1.40379 | 0.698810734 |

**Table S11.** Tissue Recombination Xenograft Weight. Supporting data for Figure 7B.

| Sample ID      | Experiment Group             | Xenograft weight (g) |
|----------------|------------------------------|----------------------|
| TRA110619-R1   | WT Epithelial + Ctrl GFP-pos | 0.0045               |
| TRA110619-R2   | WT Epithelial + Ctrl GFP-pos | 0.0021               |
| TRA110619-R4   | WT Epithelial + Ctrl GFP-pos | 0.0041               |
| TRA110619-R6   | WT Epithelial + Ctrl GFP-pos | 0.0038               |
| TRA04012020-R1 | WT Epithelial + Ctrl GFP-pos | 0.0029               |
| TRA04012020-R3 | WT Epithelial + Ctrl GFP-pos | 0.0019               |
| <b>AVERAGE</b> |                              | <b>0.0032</b>        |
| <b>S.D</b>     |                              | <b>0.0010</b>        |

| Sample ID      | Experiment Group             | Xenograft weight (g) |
|----------------|------------------------------|----------------------|
| TRA110619-R3   | WT Epithelial + ARKO GFP-pos | 0.0031               |
| TRA110619-R5   | WT Epithelial + ARKO GFP-pos | 0.0023               |
| TRA110619-R7   | WT Epithelial + ARKO GFP-pos | 0.0024               |
| TRA04012020-R2 | WT Epithelial + ARKO GFP-pos | 0.0011               |
| TRA04012020-R4 | WT Epithelial + ARKO GFP-pos | 0.001                |
| TRA04012020-R5 | WT Epithelial + ARKO GFP-pos | 0.0005               |
| <b>AVERAGE</b> |                              | <b>0.0017</b>        |
| <b>S.D</b>     |                              | <b>0.0009</b>        |

**Table S12.** Antibodies used for IHC and IF experiments in this study.

| Antibody                 | Vendors and Cat #                    | Species     | Working dilution |
|--------------------------|--------------------------------------|-------------|------------------|
| GFP                      | Cell Signaling #2956                 | rabbit IgG  | 1:200            |
| GFP                      | Cell Signaling #2955                 | mouse IgG   | 1:200            |
| GFP                      | Abcam #ab13970                       | chicken IgG | 1:2000           |
| AR                       | ThermoFisher #PA5-16750              | rabbit IgG  | 1:500            |
| Gli1                     | Novus Bio #NB600-600                 | rabbit IgG  | 1:600            |
| E-cadherin               | BD Transduction Laboratories #610182 | mouse IgG   | 1:200            |
| SMA                      | Sigma #A5228                         | mouse IgG   | 1:4000           |
| Vimentin                 | BioLegend #919101                    | chicken IgG | 1:2000           |
| Ki67                     | Cell Signaling #9129                 | rabbit IgG  | 1:500            |
| PCNA                     | Santa Cruz Biotech #sc-56            | mouse IgG   | 1:300            |
| Shh                      | Bioss Antibodies #bs-1544R           | rabbit IgG  | 1:400            |
| Nkx3.1                   | Athena Enzyme Systems #0314          | rabbit IgG  | 1:1000           |
| PBSN                     | Santa Cruz #sc-393830                | mouse IgG   | 1:200            |
| CK5                      | Covance #PRB-160P                    | rabbit IgG  | 1:2400           |
| CK8                      | BioLegend #904801                    | mouse IgG   | 1:2000           |
| CD24-APC                 | eBioscience #17-0242-80              | mouse IgG   | 1:1000           |
| CD49f-PE                 | BD Pharmigen #561894.                | rat IgG     | 1:1000           |
| Biotinylated anti-rabbit | Vector Laboratories #BA-1000         | goat IgG    | 1:750            |
| Biotinylated anti-rabbit | Vector Laboratories #BA-2000         | goat IgG    | 1:750            |
| Goat anti-rabbit 488     | Invitrogen #A11034                   | goat IgG    | 1:500            |
| Goat anti-mouse 488      | Invitrogen #A11001                   | goat IgG    | 1:500            |
| Goat anti-rabbit 594     | Invitrogen #A11012                   | goat IgG    | 1:500            |
| Goat anti-mouse 594      | Invitrogen #A11005                   | goat IgG    | 1:500            |
| Goat anti-chicken 647    | Invitrogen #A31571                   | goat IgG    | 1:200            |

**Table S13.** qPCR primer pairs used in this study.

| Genes       | Primer name | Sequences                              | Amplicon (bp) |
|-------------|-------------|--|---------------|
| <b>PP1A</b> | qPCR-PP1A-F | 5'- TGT GCC AGG GTG GTG ACT TT - 3'    | 144           |
|             | qPCR-PP1A-R | 5'- CGT TTG TGT TTG GTC CAG CAT - 3'   |               |
| <b>AR</b>   | qPCR-AR-F   | 5'- AAA CTT CTT TCG CTG GGG CTT C - 3' | 90            |
|             | qPCR-AR-R   | 5'- AGA ACA GAA CAC TAG CGC TTG G - 3' |               |
| <b>Gli1</b> | qPCR-Gli1-F | 5'- TGC CAG ATA TGC TTC AGC CA - 3'    | 290           |
|             | qPCR-Gli1-R | 5'- TGT GGC GAA TAG ACA GAG GT - 3'    |               |
| <b>Ptc1</b> | qPCR-Ptc1-F | 5'- GCA TTC TGG CCC TAG CAA TA - 3'    | 228           |
|             | qPCR-Ptc1-R | 5'- GTC TCA GGG TAG CTC TCA TA - 3'    |               |
| <b>Smo</b>  | qPCR-Smo-F  | 5'- TTG TGC TCA TCA CCT TCA GC - 3'    | 92            |
|             | qPCR-Smo-R  | 5'- TGG CTT GGC ATA GCA CAT AG - 3'    |               |