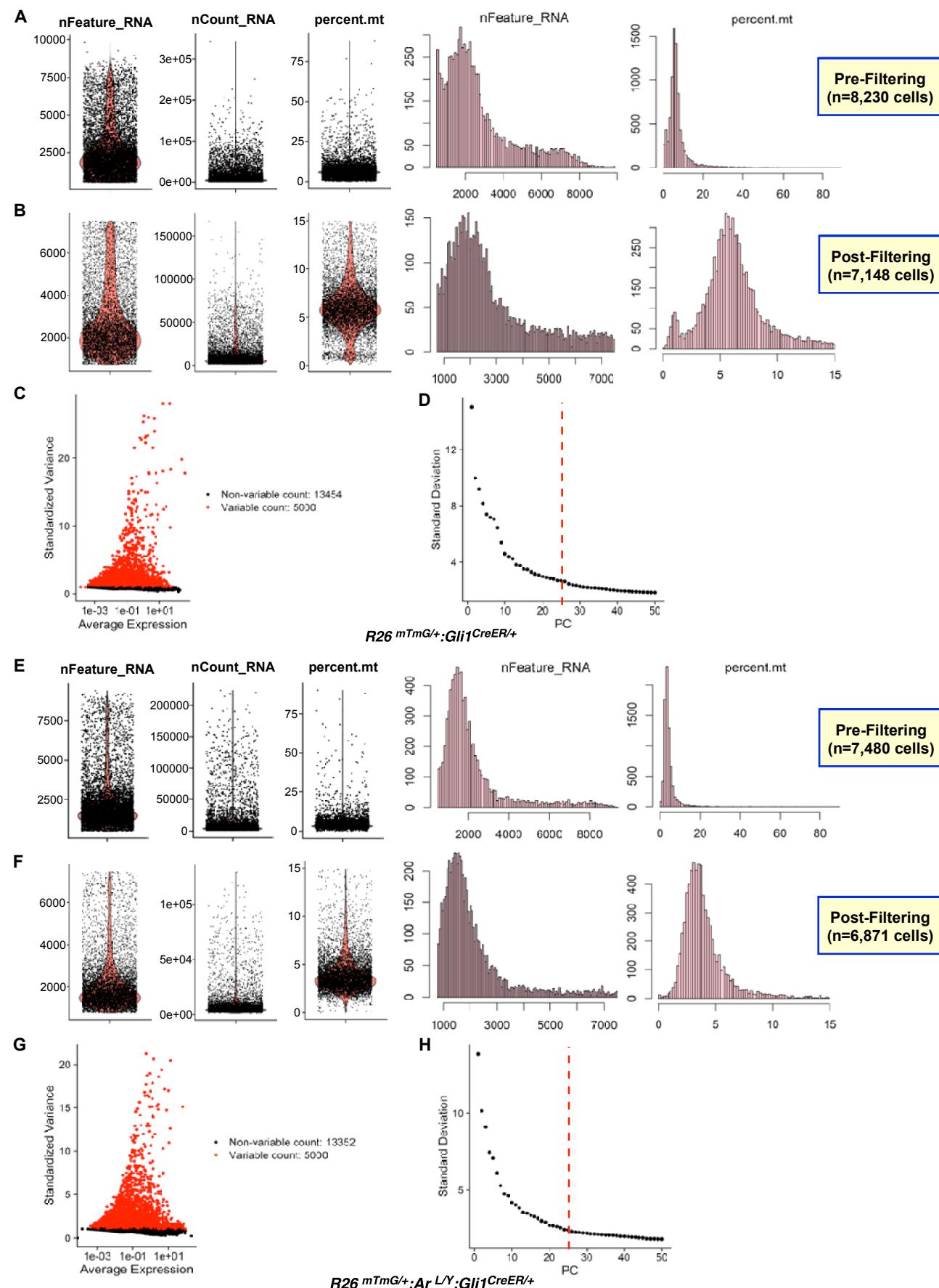
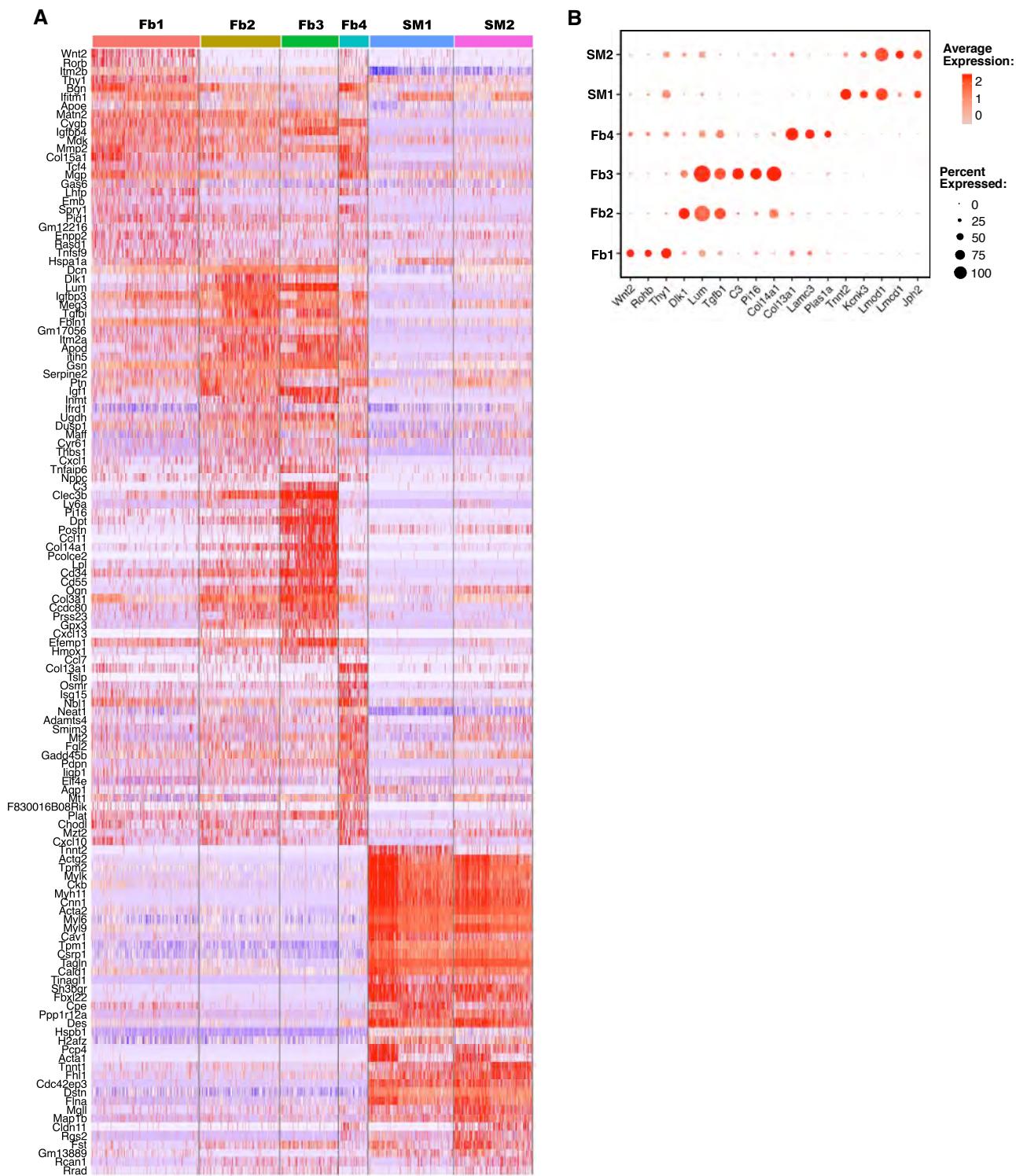


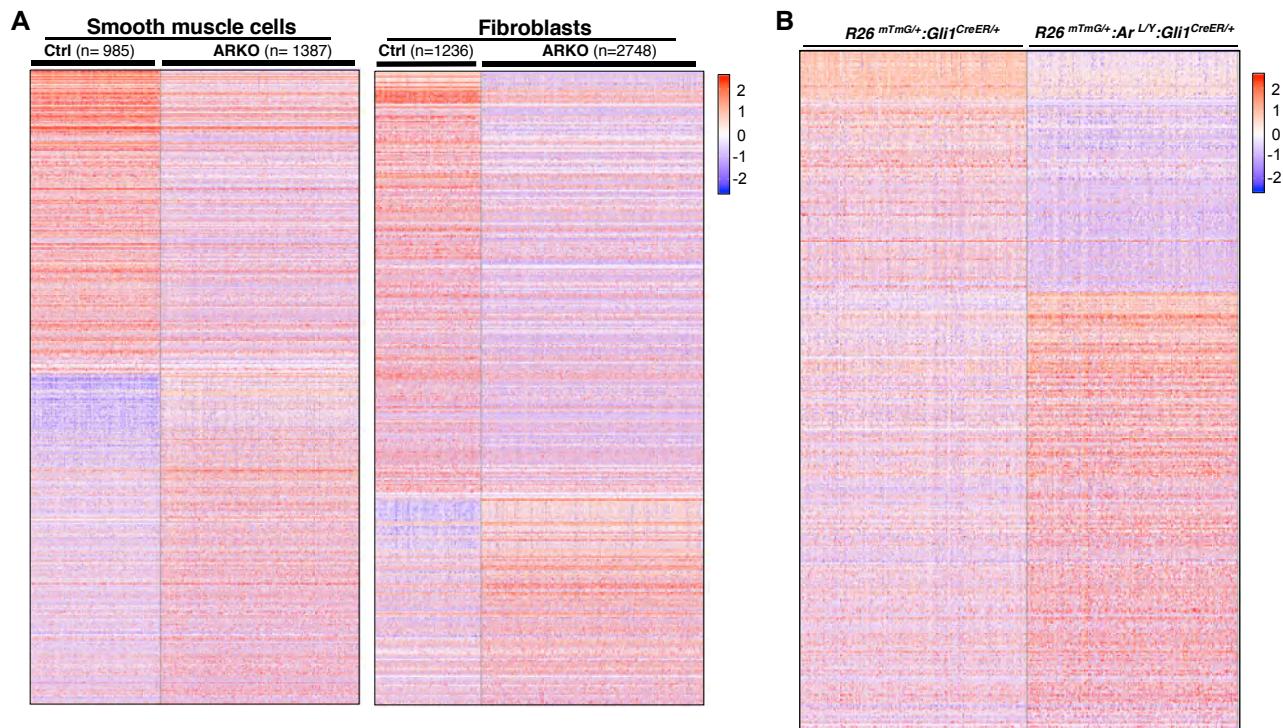
**Fig. S1. Gross and histological images of prostate tissues.** (A1-B2) Gross view images of P34 and P56 prostates collected from the indicated genotypes. (C) Ratio of prostate wet weight versus whole body weight as percentages (left) and serum testosterone concentrations (right) of mice of the indicated 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.



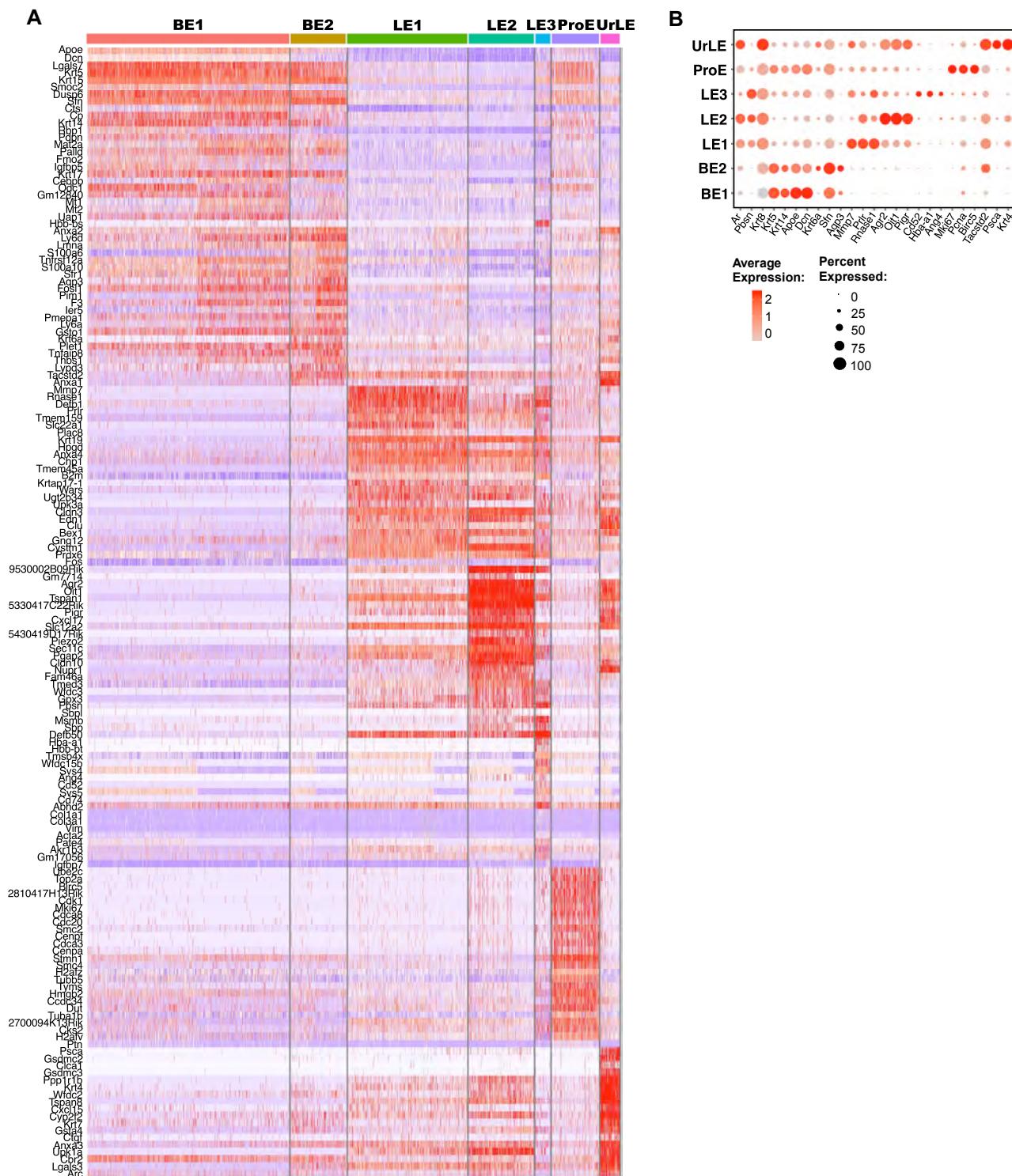
**Fig. S2. Quality controls and filtering of scRNAseq samples.** (A-B) Violin plots and histograms, visualizing the indicated metrics, pre- and post-filtering for the control sample. (C) Selection of variable genes used for principal component analysis of the control sample. (D) Elbow plot displaying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



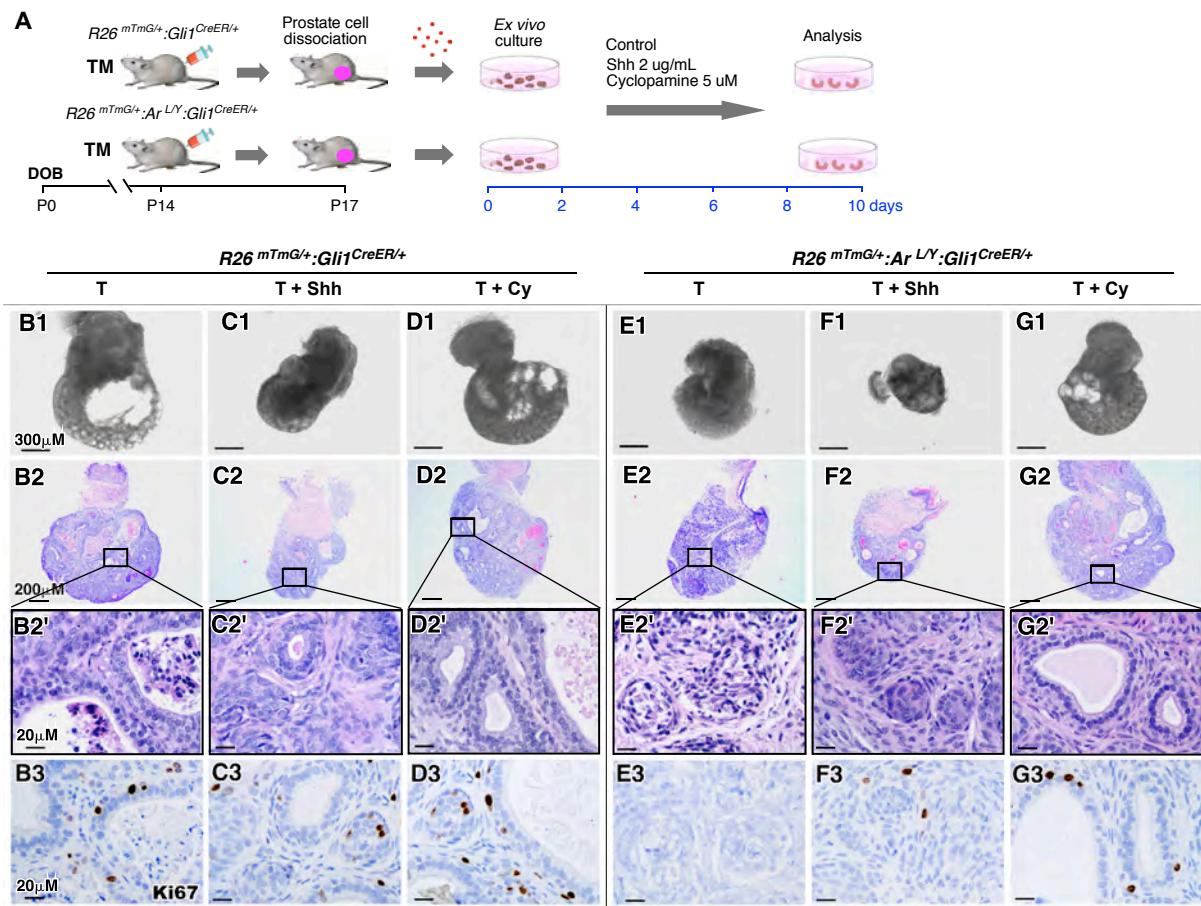
**Fig. S3. Transcriptomic profiles of prostate stromal cells.** (A) A heatmap was shown for DEGs in each of stromal cell clusters (please also see Figure 3A-D). (B) Dot plot of highly specific genes for each cellular cluster.



**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 µg/mL Shh (C1 and F1), or 5 µM 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 VS. 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 VS. 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 VS. 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 VS. 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		NES	-LOG10(FDR)
NAME			
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		NES	-LOG10(FDR)
NAME			
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'	