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Supplemental Information

Lineage Specification from Prostate Progenitor Cells Requires Gata3-Dependent Mitotic Spindle Orientation

Maxwell E.R. Shafer, Alana H.T. Nguyen, Mathieu Tremblay, Sophie Viala, Mélanie Béland, Nicholas R. Bertos, Morag Park, and Maxime Bouchard

Supplemental Information

Supplemental Figures

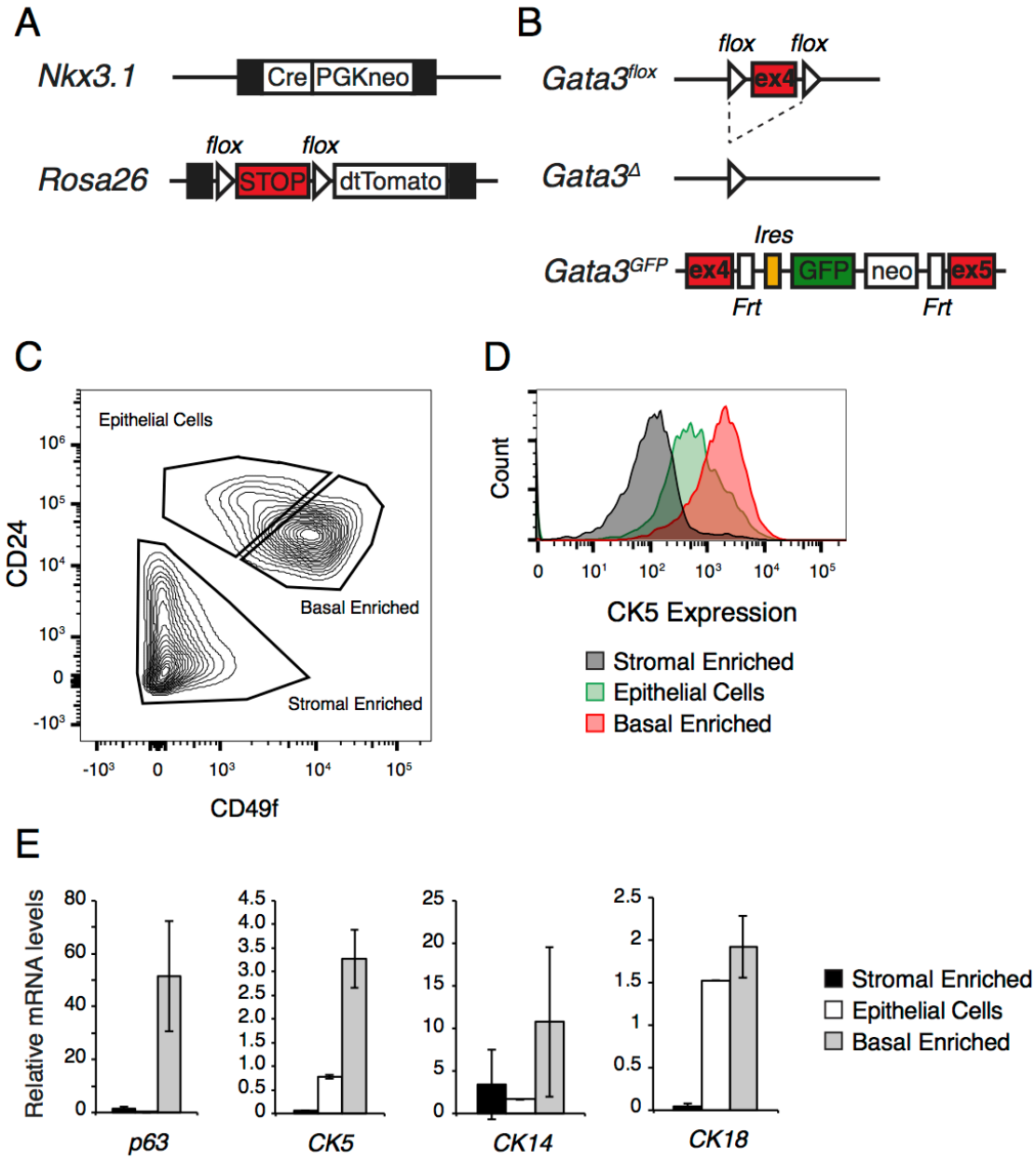

Figure S1. Lineage tracing constructs and enrichment of prostate basal cells by FACs. A) *Nkx3-1*^{Cre} was used to activate the *Rosa26*^{tdTomato} lineage tracing reporter construct. B) *Gata3* loci used in the study, including a floxed exon 4 which was removed by *Nkx3-1*^{Cre}, and an Ires GFP between exons 4 and 5 of *Gata3*. *Gata3*$^{\Delta}$ alleles were used to generate germ-line deletion mice. C) Representative fluorescent-activated cell sorting (FACS) plot of lineage negative (CD31-, Ter119-, Cd45-) cells from 2 week old prostate tissue showing stromal, epithelial and basal enriched cell populations based on CD24 and CD49f expression levels. D) Expression levels of CK5 in populations identified in C as measured by FACS. E) qRT-PCR of basal and luminal markers in FACS sorted populations. Error bars represent standard deviation from 3 technical replicates. FACS plots are representative of experiments from 3 individual prostates. See also Figure 1.

Figure S2. *Gata3*-deficient prostate branching defects are persistent in sexually mature mice. A) Ductal architecture of anterior, dorsal-lateral and ventral lobes from control and *Nkx3-1*^{Cre}*; Gata3*$^{flox/flox}$ prostates at 6 weeks old. Scale bar equals 1mm. See also Figure 2.

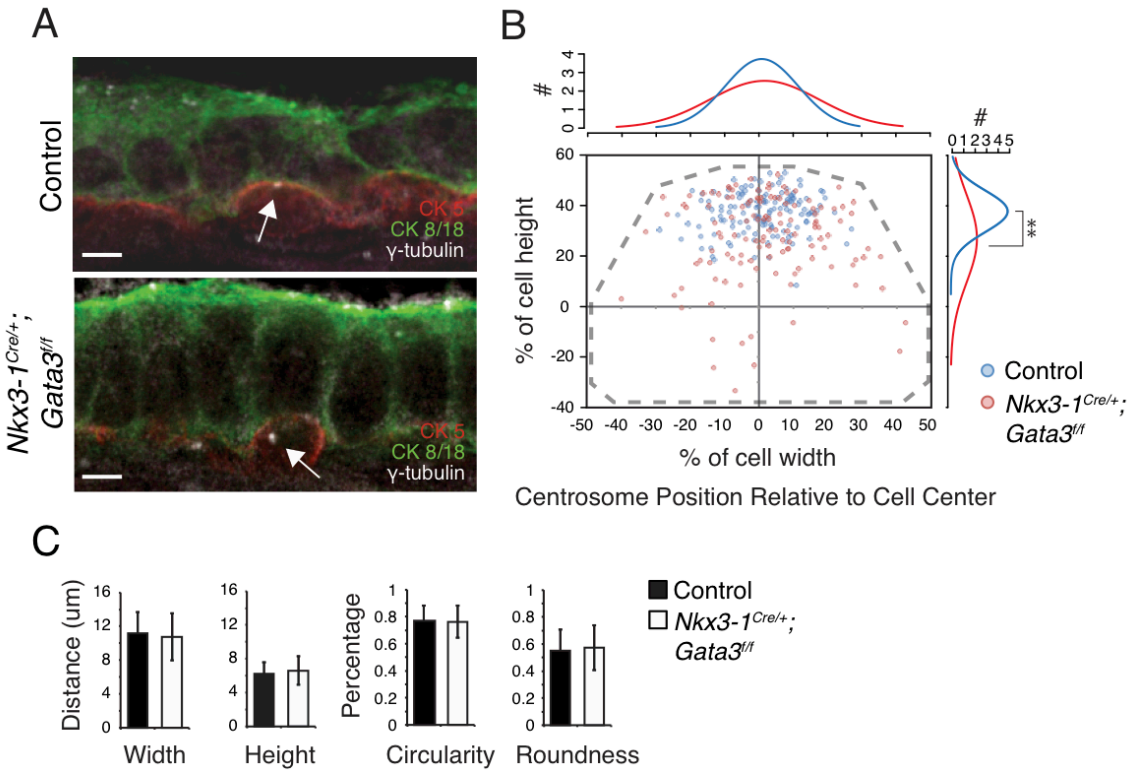

Figure S3. *Gata3* controls interphase centrosome localization in prostate progenitor cells. A) Localization of centrosomes (arrows) in control and *Nkx3-1*^{Cre}*; Gata3*$^{flox/flox}$ double positive progenitor cells by γ-tubulin immunofluorescence. Scale bars equal 5μm. B) Quantification of centrosome location relative to the centre of each cell in control and *Nkx3-1*^{Cre}*; Gata3*$^{flox/flox}$ progenitor cells. Location of each centrosome was represented as a percentage of the cell height or width. C) Size and shape of prostate progenitor cells in absence or presence of *Gata3*. Quantification is from 3 control and 3 *Nkx3-1*^{Cre}*; Gata3*$^{flox/flox}$ prostates**.** See also Figure 4.

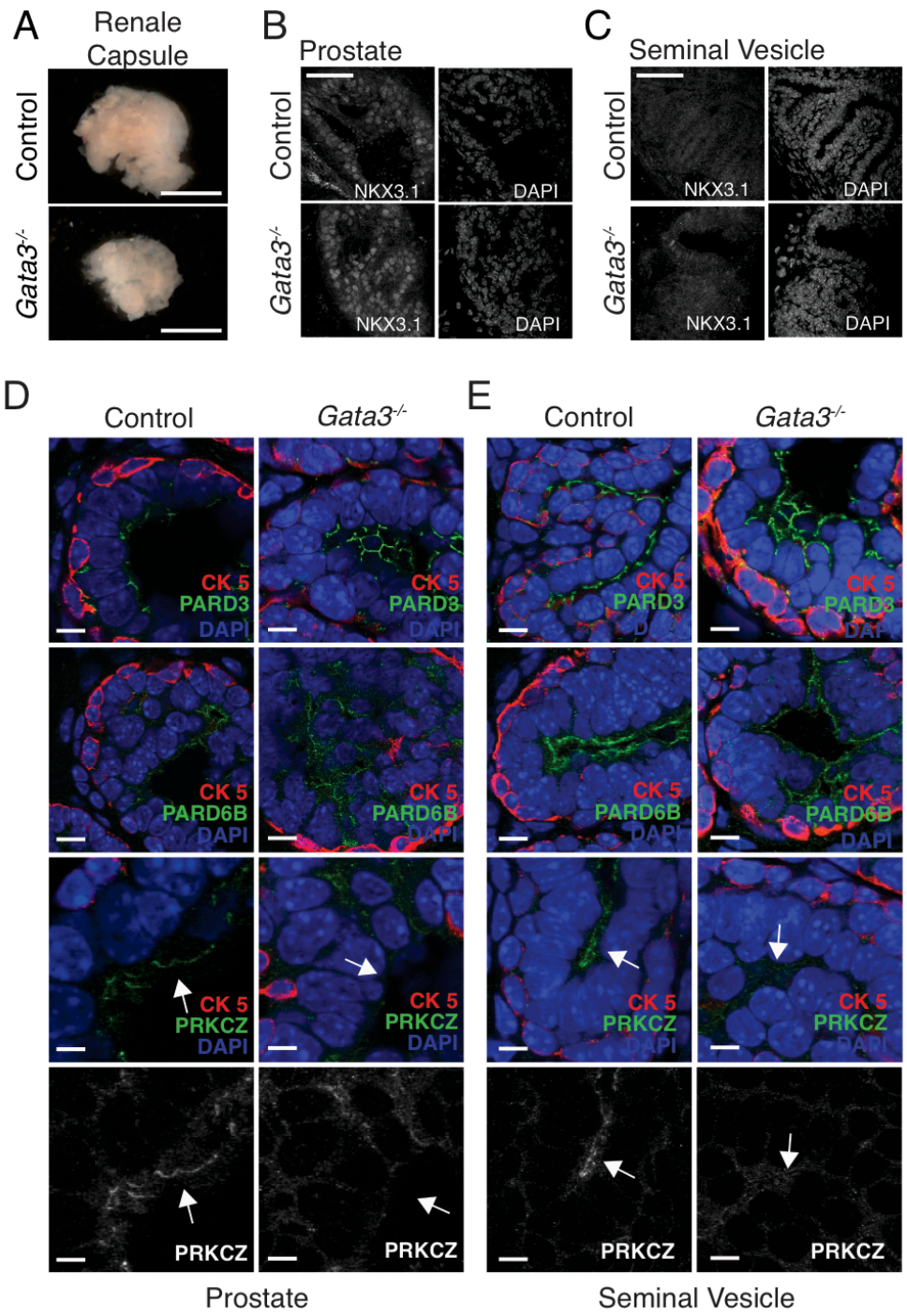

Figure S4. Germline deletion of *Gata3* affects prostate and seminal vesicle tissue. A) Urogenital tissue from drug rescued germline *Gata3* knockout and control embryos were grown under the kidney capsule of immunodeficient mice for 14 days. Scale bars are equal to 1mm. Presence of prostate (B) and seminal vesicle (C) after growth under the kidney capsule as detected by Nkx3-1 immunofluorescence staining and epithelial histology. Scale bars are equal to 50µm. D) Expression and localization of Pard3, Pard6b and aPKCz/i in drug rescued prostate tissue of germline *Gata3* knockout embryos grown under the kidney capsule. E) Expression and localization of Pard3, Pard6b and aPKC in drug rescued germline *Gata3* knockout grafted seminal vesicle tissue. Scale bars are equal to 10 µm. See also Figure 3. Arrows indicate the apical membrane.

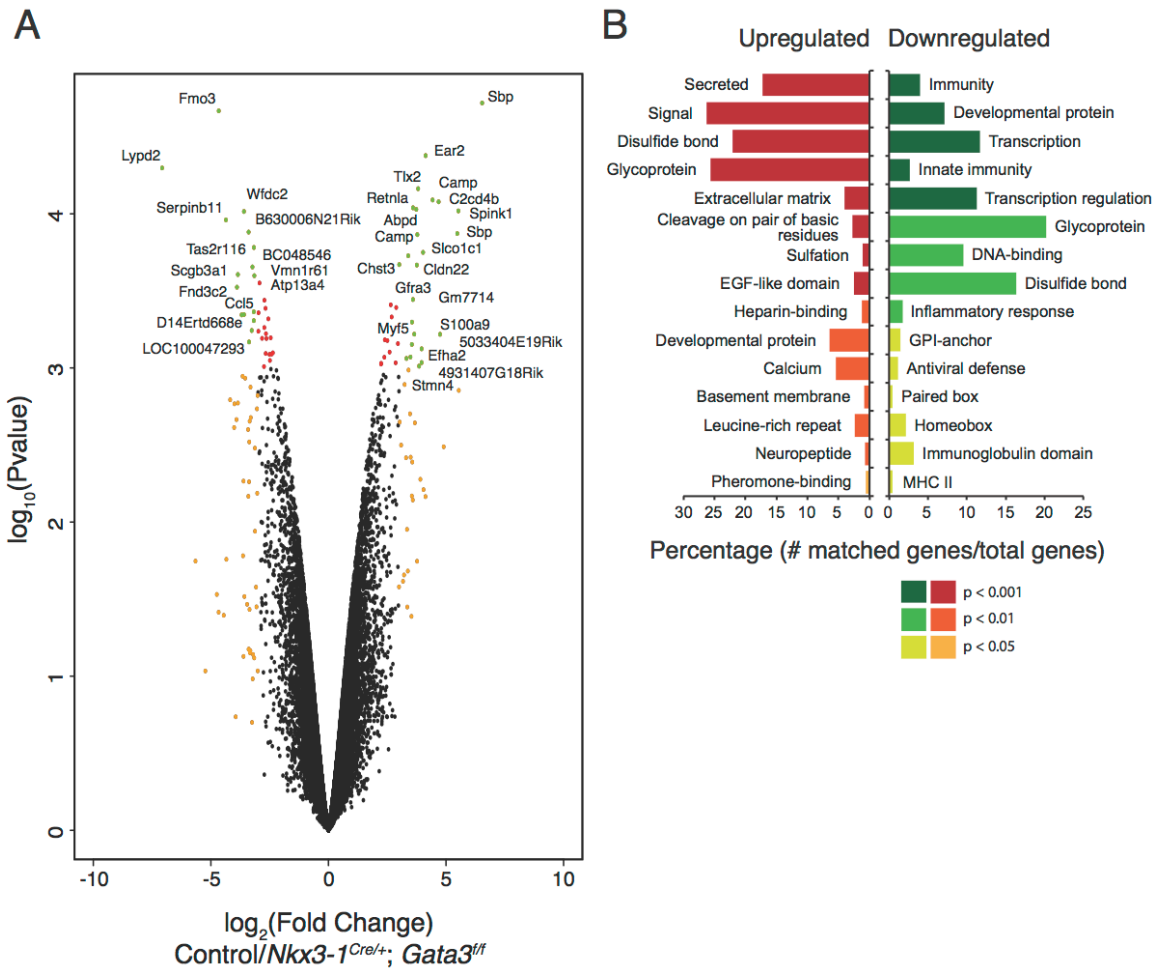

Figure S5. Transcriptional consequences of Gata3 deficiency. Microarray analysis was performed on epithelial cells obtained by laser capture micro dissection from 3 control and 3 *Nkx3-1*^{Cre}*; Gata3*$^{flox/flox}$ prostates. A) Volcano plot of transcripts differentially expressed between control and *Nkx3-1*^{Cre}*; Gata3*$^{flox/flox}$ prostate tissue. Colours represent transcripts which are log2(Fold Change)>3 (orange) or p-value<0.001 (red) or both (green, text labels). Labelled transcripts are also listed in Supplemental Table 1. B) Analysis of the differentially expressed transcripts for GO term enrichment using functional categories. Analysis was done using the most recent release of DAVID.

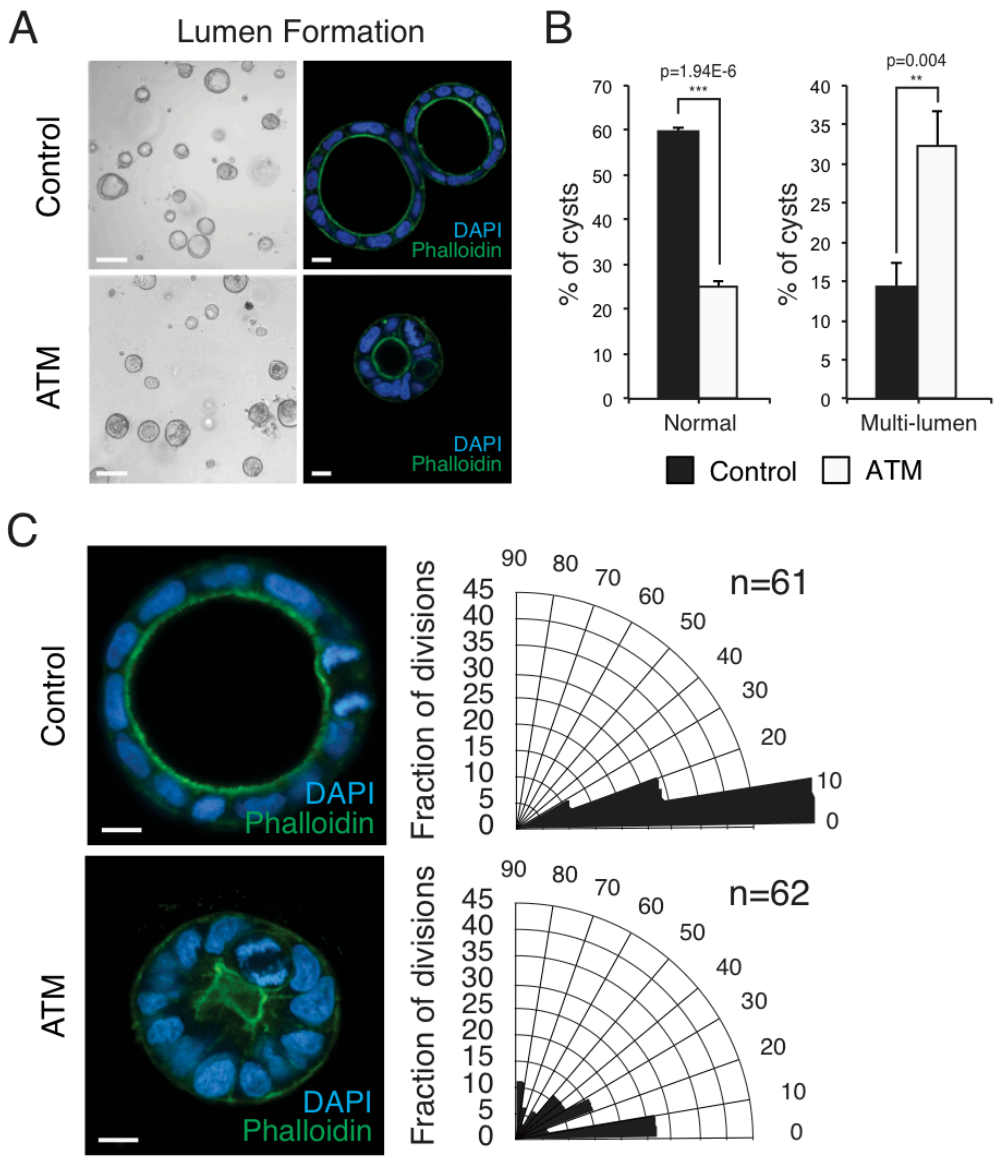

Figure S6. Mis-localization of aPKC causes epithelial defects in Caco-2 cysts. A) Effect of ATM on lumen formation in epithelial cysts by light microscopy and immunofluorescence using phalloidin and DAPI staining. Scale bars equal 100μm for bright field and 5μm for IHC. B) Quantification of normal or multi-lumen cysts after treatment with ATM. Error bars represent standard deviation of 3 independently treated samples of Caco-2 cysts. C) Measurement of spindle orientation in control and ATM-treated Caco-2 epithelial cysts. Spindle orientation was measured relative to the basal side of the cyst using DAPI and phalloidin staining. Scale bars are equal to 5μm. N represent the number of cell divisions measured. See also Figure 4.

Supplemental Table 1. Top regulated genes by microarray analysis of *Gata3* deficient epithelium ($(\log_2(\text{fold change}))>3$, p-value < 0.001).

Probe name	GeneSymbol	log2(Fold change)	P-value
A_55_P2078153	Sbp	6.535852	1.91E-05
A_51_P365516	Spink1	5.525317	9.58E-05
A_52_P294663	Sbp	5.479108	0.000134287
A_55_P1998471	S100a9	4.746343	0.000604859
A_55_P2054261	C2cd4b	4.686068	8.36E-05
A_51_P505521	Hist1h4i	4.449953	3.06E-05
A_55_P2039699	Camp	4.430271	8.13E-05
A_55_P1952618	Ear2	4.13247	4.19E-05
A_55_P2185832	Slco1c1	4.025487	0.000177908
A_55_P2428514	Retn	3.960925	0.000922969
A_51_P188281	Myf5	3.960323	0.000751629
A_55_P2045642	Stmn4	3.855307	0.000973621
A_55_P2164659	Tlx2	3.814866	6.87E-05
A_55_P2058201	Scgb2b20	3.780043	0.000136517
A_55_P2112459	Cldn22	3.758181	0.000215143
A_55_P2040653	Gm10824	3.73503	9.36E-05
A_51_P188281	Myf5	3.649437	0.000603499
A_51_P257951	Retnla	3.594639	9.14E-05
A_55_P1984103	Gfra3	3.592741	0.000359646
A_55_P2062078	Gm7714	3.553273	0.000504486
A_55_P2350665	5033404E19Rik	3.547267	0.000703888
A_55_P2007851	Micu3	3.487156	0.000849639
A_55_P2158102	Camp	3.392757	0.00018712
A_51_P127320	Cabs1	3.318	0.000867584
A_55_P2098067	Etos1	-3.151594	0.000252018
A_55_P1965574	BC048546	-3.169077	0.000165391
A_52_P638459	Ccl5	-3.174343	0.000493041
A_55_P2038217	Atp13a4	-3.174746	0.00043225
A_51_P445320	Tas2r116	-3.232771	0.000221261
A_55_P2015687	Phf11d	-3.263846	0.000571518
A_55_P2132982	Zfp987	-3.376576	0.000677995
A_55_P2421067	B630006N21Rik	-3.394777	0.000131393
A_55_P1972169	Vmn1r61	-3.593272	0.000450544
A_55_P1953341	Wfdc2	-3.59373	9.66E-05
A_55_P2033947	Fnd3c2	-3.691144	0.000452706
A_66_P130759	4631405J19Rik	-3.850394	0.000247762
A_51_P110341	Scgb3a1	-3.890031	0.000299347
A_51_P438853	Serpinb11	-4.35963	0.000109572
A_51_P269404	Fmo3	-4.664987	2.15E-05
A_51_P136521	Lypd2	-7.07352	5.04E-05

Supplemental Table 2. Primers used in this study

Gene	Usage	Forward Primer	Reverse Primer
Gata3 +/f/Δ	Genotyping	GTCAGGGCACTAAGGGTTGTT	TGGTAGAGTCCGCAGGCATTG
		TATCAGCGGTTCATCTACAGC	
Nkx3-1 Cre/+	Genotyping	GCG CGG TCT GGC AGT AAA AAC	CAG ATG GCG CGG CAA CAC C
R26 LacZ/+	Genotyping	ATACTGTCGTCGTCCCCTCAAACTG	TTCAACCACCGCACGATAGAGATT
Gata3 (exon 4)	qRT-PCR	TTATCAAGCCCAAGCGAAG	TGGTGGTGGTCTGACAGTTC
p63 (Tpr63)	qRT-PCR	TCGATGTGTCCTTCCAGCAGTCAA	TGTAGACAGGCATGGCACGGATAA
CK5 (Krt5)	qRT-PCR	GAGATCGCCACCTACAGGAA	TCCTCCGTAGCCAGAAGAGA
CK14 (Krt14)	qRT-PCR	CCTCTGGCTCTCAGTCATCC	CCTCTGGCTCTCAGTCATCC
CK18 (Krt18)	qRT-PCR	CGAGGCACTCAAGGAAGAAC	AATCTGGGCTTCCAGACCTT

Supplemental Table 3. Antibodies used in this study

Antibody	Source	Dilution
CK5 (Chicken)	Covance, SIG-3475	1:200
CK8 & CK18 (Guinea Pig)	Fitzgerald, 20R-CP004	1:200
Gata3 (Rabbit)	Santa Cruz, sc-9009	1:50
E-cadherin (Mouse)	BD Biosciences	1:200
ZO-1 (Rat)	Chemicon	1:200
Phospho-H3 (Rabbit)	Millipore, 06-570	1:100
γ-tubulin (Goat)	Santa Cruz, sc-7396	1:100
Pard3 (Rabbit)	Millipore, 07-330	1:100
Pard6b (Rabbit)	Santa Cruz, sc-67393	1:50
aPKCz/i (Rabbit)	Santa Cruz, SC-216	1:50
Nkx3-1 (Rabbit)	Santa Cruz, sc-25406	1:50
Phalloidin	Life Technologies	1:500

Supplemental Table 4. Spindle orientation chi-squared test results

Condition 1	Condition 2	Chi-squared p-value	Level of significance	Figure
All Cells (Control)	All Cells (Nkx3-1Cre;Gata3f/f)	<0.001	****	2E
Basal Cells (Control)	Basal Cells (Nkx3-1Cre;Gata3f/f)	<0.001	****	2E
Luminal Cells (Control)	Luminal Cells (Nkx3-1Cre;Gata3f/f)	<0.001	****	2E
Double Positive Cells (Control)	Double Positive Cells (Nkx3-1Cre;Gata3f/f)	<0.001	****	2E
Caco2 (Control)	Caco2 (ATM Treated)	<0.001	****	4A
All Cells (Control)	All Cells (ATM Treated)	<0.001	****	4C
Basal Cells (Control)	Basal Cells (ATM Treated)	0.0278	*	4C
Luminal Cells (Control)	Luminal Cells (ATM Treated)	<0.001	****	4C
Double Positive Cells (Control)	Double Positive Cells (ATM Treated)	0.2468	ns	4C

Supplemental Materials and Methods

Drug rescue and kidney capsule implantation

To generate germline *Gata3* mutant urogenital tissue, *Gata3*$^{-/-}$ embryos, which normally die around E11.5, were grown until E18.5 using a modified version of a drug rescue regimen (Kaufman et al., 2003; Lim et al., 2000). E5.5-E7.5, pregnant *Gata3*$^{+/-}$ dams were given fresh water daily containing 100 μg/ml isoproterenol, 1 mg/ml DOPA and 2 mg/ml ascorbic acid in a light protected container (Sigma). E18.5 male urogenital systems (including the urethra, seminal vesicles and prostate) were then transferred under the kidney capsule of a male immunodeficient mouse with a sub-cutaneous testosterone plug for 14 days as previously described (Nicholson et al., 2013).

Laser capture microdissection and microarray analysis

Prostate epithelial cells were isolated by laser capture micro-dissection of cryo-sectioned 2-week old prostates. Epithelial ducts were captured by infrared laser using an ArcturusXT Microdissection System (Applied Biosystems). RNA was extracted and subjected to two rounds of linear amplification before labeling and hybridization to Agilent microarray chips. Microarray chips were scanned on a Microarray Scanner Model G2505B (Agilent Technologies).

Microarray data were normalized, and differential expression was performed using the LIMMA package in FlexArray (Genome Quebec). The volcano plot was generated using R Studio and GO term enrichment was done using the most recent release of DAVID (6.8 Beta) (Huang da et al., 2009a, b).

Aurothiomalate administration in vitro

Caco-2 cells were grown in DMEM (10% FBS) (Gibco) with appropriate antibiotics. Cysts were produced by plating Caco-2 cells in matrigel (Corning) and grown for 7-10 days. For inhibition of the aPKC-Par6b interaction, aurothiomalate (ATM) was added to the media for the duration of cyst growth.

Image analysis

Proliferation/apoptosis, spindle orientation analysis, cell lineage quantification, basal cell centriole localization, and aPKC fluorescence intensity were all and analyzed using Fiji (ImageJ) software. Proliferation/apoptosis and cell lineage quantification was performed using the Cell Counter plugin from representative fields of view (Fiji). DAPI positive cells were first identified, then each was assigned as CK5+, CK8/18+ or double positive. Counts were standardized for the circumference of the each duct quantified. Centriole localization was performed by first straightening the prostate duct along the basal side using Fiji, followed by calculation of the size, position and shape of both the basal cells and their centrioles.

Statistical analysis

For differences in proliferation, apoptosis, number of cells per ductal boundary and centrosome location, statistical significance was determined by student's t-test. Statistical analysis of spindle orientation was determined by chi-squared analysis as previously described (Williams et al., 2014), by separating spindle orientations measurements into 3 bins; 0-20°, 20-70° or 70-90°. Statistical analyses were performed using Prism software (GraphPad).

Supplementary References

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