Sonic hedgehog signals to multiple prostate stromal stem cells that replenish distinct stromal subtypes during regeneration

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The adult mouse prostate has a seemingly endless capacity for regeneration, and sonic hedgehog (SHH) signaling has been implicated in this stem cell-driven process. However, it is not clear whether SHH acts on the epithelium or stromal cells that secrete factors required for epithelial expansion. Because little is known about stromal stem cells compared with their epithelial counterparts, we used *in vivo* mouse genetics tools to characterize four prostate stromal subtypes and their stem cells. Using knockin reporter alleles, we uncovered that SHH signals from prostate basal epithelial cells to adjacent stromal cells. Furthermore, the SHH target gene *Gli1* is preferentially expressed in subepithelial fibroblast-like cells, one of four prostate stromal subtypes and the subtype closest to the epithelial source of SHH. Using Genetic Inducible Fate Mapping to mark adult *Gli1*- or *Smooth muscle actin*-expressing cells and follow their fate during regeneration, we uncovered that *Gli1*-expressing cells exhibit long-term self-renewal capacity during multiple rounds of androgen-mediated regeneration after castration-induced involution, and depleted smooth muscle cells are mainly replenished by preexisting smooth muscle cells. Based on our Genetic Inducible Fate Mapping studies, we propose a model where SHH signals to multiple stromal stem cells, which are largely unipotent *in vivo*.

genetic fate mapping | mesenchymal lineage analysis | smooth muscle | *Gli1* expression

In self-renewing organs, sonic hedgehog (SHH) signaling regulates adult stem cells, including neural stem cells in the forebrain (1, 2) and stem cells in the hair follicle (3, 4). However, it is unclear whether SHH signaling also regulates stem cells in normally quiescent organs, such as the prostate. Because the murine prostate can maintain its size after 15 rounds of castration-induced involution and androgen-mediated regeneration (5, 6), at least the epithelial compartment is driven by stem cells. During regeneration, both the epithelium and stroma must be replenished, possibly by separate stem cells. Given that stem cells likely maintain tissue homeostasis and that their deregulation can result in diseases, it is important that dormant adult stromal stem cells in the prostate are identified.

The prostate consists of epithelial ducts made up of three cell types (basal, luminal, and neuroendocrine) surrounded by stromal tissue mainly made up of smooth muscle cells (SMCs) and interstitial fibroblasts. In mouse, the prostatic ducts resemble tree-like structures, and each duct has a proximal region that connects to the urethra. Multiple studies have shown that the proximal region is enriched for epithelial stem cells, with a high capacity to expand *in vitro* and reconstitute prostate tissue when mixed with embryonic urogenital sinus mesenchyme and grafted under the kidney capsule (6–8). *In vivo* Genetic Inducible Fate Mapping (GIFM) studies further have shown that luminal and basal cells are mainly generated by lineage-restricted unipotent stem cells during regeneration in adult mouse prostate (9–11). However, whether there is a separate source of stem cells to replenish the stromal compartment and whether they are unipotent

in vivo during regeneration have not been addressed. Given that stromal cells play an essential role in maintaining the epithelium during prostate development (12) and modify prostate benign and malignant disease progression (13, 14), it is critical that the cellular processes underlying regeneration of the stromal compartment be determined.

In mammals, the HH ligands act through multiple transmembrane proteins, including smoothened (SMO) (15, 16). SMO activation promotes the formation of GLI2 and GLI3 activators, which induce expression of target genes, including a third *Gli* gene *Gli1*. Because *Gli1* transcription is dependent on GLI2 and GLI3 activators, *Gli1* expression is a sensitive readout of high-level HH signaling (17, 18). GLI1 functions as a feed-forward activator of the HH signaling pathway, but *Gli1* is dispensable for mouse development, including development of the prostate (17, 19, 20).

Shh expression diminishes postnatally in the mouse prostate, but it is maintained at low levels throughout adulthood (21, 22). During embryogenesis, *Shh* is expressed by urogenital sinus epithelium, and *Gli1* is expressed in adjacent mesenchyme, indicating unidirectional signaling from the epithelium to the mesenchyme (22). *Shh* is required for prostate development within the tissue and to stimulate androgen production (22–24), and interestingly, SHH signaling exerts stage-specific effects in the developing prostate (21, 25, 26). The cell types that are responsive to SHH signaling in the adult prostate are currently

Significance

Sonic hedgehog (SHH) signaling regulates adult stem cells in many self-renewing organs. However, whether SHH also regulates adult stem cells in tissues like the prostate is unclear. Improving therapy for prostate diseases, including benign prostatic hyperplasia and prostate cancer, will require a better understanding of the key signaling pathways that regulate prostate homeostasis and regeneration. We show that SHH signals from basal epithelial cells to the surrounding stroma in the adult prostate. Moreover, SHH responding cells repopulate the stroma and exhibit long-term self-renewal capacity during multiple rounds of involution and regeneration. Furthermore, we identified four stromal subtypes and present evidence for a model in which the three ductal stromal subtypes are maintained largely by distinct unipotent stem/progenitor cells.

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Fig. 2. *Gli1* expression is enriched in prostate ductal subepithelial cells. (A) FIHC staining of DP sections for SMA (green) and CD34 (red) showing four stromal subtypes: subepithelial cell (Sub), SMC, wrapping cell (Wrap), and interstitial fibroblast (IF). (B) FIHC staining of *Gli1*^{lacZ/+} DP sections for SMA (green) and bGAL (red) showing that *Gli1* is expressed in Sub, SMC, and Wrap. (C and D) Percentage of *Gli1*⁺ cells in the three ductal stromal subtypes compared with the normal distribution of each subtype as a percentage of CD34⁺ cells; 10,159 CD34⁺ cells (including *Gli1*⁺ cells) were counted from three mice. Data are presented as mean $\pm$ SEM. ** $P < 0.01$; *** $P < 0.001$ by ANOVA. (Scale bar: 20 μ m.)

respectively, $P > 0.05$). Thus, *Gli1* is preferentially expressed by the stromal ductal subtype in closest proximity to the epithelial source of SHH (subepithelial cells).

***Gli1*-Expressing Cells and Their Progeny Expand During Regeneration After Castration-Induced Involution.** In self-renewing adult tissues, our previous studies using in vivo GIMF showed that *Gli1*-expressing cells are quiescent stem cells that are maintained for the life of the mouse and give rise to transit-amplifying cells that expand and produce olfactory bulb interneurons (2) or hair follicle epithelium (4). We, therefore, used a similar approach to test whether *Gli1*-expressing cells in the adult prostate are maintained and replenish the stroma. A prerequisite for such a study is the presence of cell turnover in the stroma of the adult prostate. Previous studies found evidence for cell death in the stroma of the ventral prostate using TUNEL (34) and EM (35). Because our study is focused on the DP that has the greatest amount of stroma, we performed TUNEL staining on DP sections 7 d after castration. We found evidence for cell death in all three ductal stromal subtypes during involution, with more evidence in nonproximal regions (proximal: 8.4% of subepithelial cells, 1.5% of SMCs, and 14.6% of wrapping cells; nonproximal: 15.3% of subepithelial cells, 5.7% of SMCs, and 17.4% of wrapping cells) (Fig. S3). Next, we examined the extent of cell proliferation in the three stromal subtypes in the DP 3 d into regeneration and found that the three stromal subtypes displayed proliferation, with more distally (proximal: 6.9% of subepithelial cells, 1.4% of SMCs, and 1.6% of wrapping cells; nonproximal: 10.5% of subepithelial cells, 1.5% of SMCs, and 3.0% of wrapping cells) (Fig. S3). Thus, stromal cells in the DP undergo extensive turnover during involution/regeneration (I/R).

Adult *Gli1*-expressing cells were marked and fate mapped by administering Tamoxifen to *Gli1*^{CreER/+}; *R26*^{lacZ/lacZ} males, and the population of cells marked by *Gli1*-GIMF was compared in prostates of mice that had or had not undergone I/R (Fig. 3A). The dose of Tamoxifen was adjusted such that only a few cells per section were initially labeled (defined as 5 d after Tamoxifen treatment and referred to as 5 d non-I/R). Significantly, the percentage of the prostate ductal area labeled after one round of I/R was ~13-fold higher than in 5 d non-I/R controls ($19.1 \pm 4.5\%$ vs. $1.4 \pm 0.4\%$, $P < 0.01$) (Fig. 3B, D, and F). Moreover,

cells marked by *Gli1*-GIMF were restricted to the stroma and did not contribute to the epithelium (Fig. 3D), which was confirmed by double FIHC labeling of YFP and CD34 in *Gli1*^{CreER/+}; *R26*^{Yfp/Yfp} prostates (Fig. S4). Thus, HH signaling does not induce *Gli1* transcription in rare epithelial stem cells; instead, it preferentially signals to stromal cells that can repopulate the prostatic stroma after I/R.

To examine the rate of cell turnover in intact homeostatic prostates, we compared non-I/R animals that were killed 5 d (5 d non-I/R) or 5 wk (5 wk non-I/R) after Tamoxifen administration. We found a trend to an increase, albeit not significant, in labeled area from 5 d to 5 wk non-I/R ($1.4 \pm 0.4\%$ vs. $5.7 \pm 1.3\%$, $P > 0.05$) (Fig. 3B, C, and F). However, the X-GAL⁺ area in I/Rx1 significantly expanded approximately threefold compared with 5 wk non-I/R ($19.1 \pm 4.5\%$ vs. $5.7 \pm 1.3\%$, $P < 0.05$) (Fig. 3C, D, and F).

To study the long-term self-renewal capacity of cells marked by *Gli1*-GIMF, we subjected *Gli1*^{CreER/+}; *R26*^{lacZ/lacZ} mice to three rounds of I/R; we found that the labeled cells persisted and that the percentage of labeled area was similar to the percentage of labeled area of I/Rx1 ($17.6 \pm 3.1\%$, $P > 0.05$) (Fig. 3D–F). This result shows that the cells marked by *Gli1*-GIMF can maintain a steady cell population over a period of extensive cell turnover. To further test this conclusion, we subjected mice to 10 and 12 rounds of I/R, and the cells marked by *Gli1*-GIMF still persisted (Fig. S4), indicating that *Gli1*-expressing cells include

Fig. 3. *Gli1*-GIMF-labeled dorsal prostate stromal cells expand after one round of I/R. (A) Schematic showing experimental design; 5 d non-I/R and 5 wk non-I/R indicate non-I/R mice that were killed 5 d and 5 wk after being administered Tamoxifen, respectively. (B–E) X-GAL staining of *Gli1*^{CreER/+}; *R26*^{lacZ/lacZ} DP sections from mice after (B) 5 d non-I/R, (C) 5 wk non-I/R, (D) one round of I/R (I/Rx1), and (E) three rounds of I/R (I/Rx3). (F) Quantification of the percentage of ductal stromal area that is X-GAL⁺ in *Gli1*^{CreER/+}; *R26*^{lacZ/lacZ} DPs. * $P < 0.05$; ** $P < 0.01$ by ANOVA. Data are presented as mean $\pm$ SEM. Each data point represents the average of three sections from one animal. (Scale bar: 100 μ m.)

stromal stem/progenitor cells that have the capacity for long-term self-renewal.

Our *Gli1*-GIFM results, which show an expansion of *Gli1*-expressing cells after I/R, could mean that either *Gli1* is preferentially expressed in stromal stem/progenitor cells or all stromal cells can expand the stroma. To distinguish between these possibilities, we randomly marked stromal cells using an *R26^{CreER/lacZ}* allele (epithelial cells were also marked but excluded from the quantification). Interestingly, we found that randomly marked prostate stromal cells did not expand significantly in prostates that had undergone one round of I/R compared with 5 d non-I/R control DPs ($1.9 \pm 0.8\%$ vs. $3.1 \pm 2.8\%$, $P > 0.05$) (Fig. S5). This result indicates that most randomly marked stromal cells can, at best, maintain the prostate stroma. In sharp contrast, *Gli1*-expressing cells preferentially expand the prostatic stroma during the first round of I/R.

***Gli1*-Expressing Cells Preferentially Expand the SMCs in the Nonproximal Regions of Prostatic Ducts During Regeneration.** Given that *Gli1* marks stromal stem/progenitor cells, we examined which specific subtypes are marked with *Gli1*-GIFM after regeneration using *Gli1^{CreER/+}; R26^{Yfp/Yfp}* mice. In the proximal regions of prostatic ducts, both the cell number and the proportion of each YFP+ subtype were not significantly different between I/Rx1 and 5 wk non-I/R prostates (Fig. 4D). Interestingly, in the nonproximal regions, we found that the number of SMCs marked by *Gli1*-GIFM was increased nearly threefold in I/Rx1 DPs compared with 5 wk non-I/R DPs (349 ± 90 vs. 136 ± 19 cells/mm² of SMA+ area, $P < 0.001$) (Fig. 4F). In contrast, the number of subepithelial cells and wrapping cells did not change significantly. The total number of labeled YFP+ cells increased approximately twofold in I/Rx1 DPs compared with in 5 wk non-I/R DPs (544 ± 105 vs. 256 ± 15 cells/mm² SMA+ area, $P < 0.05$). The approximately threefold increase in X-GAL area (above) is likely caused by the larger cell size of the SMC subtype of stroma and its preferential expansion after regeneration. In conclusion, SMCs marked by *Gli1*-GIFM preferentially expand only in the nonproximal regions of prostatic ducts after I/R, whereas subepithelial and wrapping cells are maintained.

Because we observed different levels of cell death in the three ductal stromal subtypes 7 d into involution, we examined the distribution of stromal subtypes marked by *Gli1*-GIFM in *Gli1^{CreER/+}; R26^{Yfp/Yfp}* DPs 14 d after castration (end of I). Significantly, the

percentage of labeled SMCs among all three labeled subtypes was decreased in the end of I DPs ($31.8 \pm 4.8\%$ vs. $53.3 \pm 7.3\%$ of total YFP+ cells, $P < 0.05$) (Fig. 4D and E), indicating that labeled SMCs are preferentially depleted over the full course of involution. In contrast, the percentage of labeled subepithelial cells and wrapping cells did not change significantly. Finally, both the number and the proportion of labeled SMCs were greatly increased in I/Rx1 DPs compared with end of I DPs (349 ± 90 vs. 65 ± 11 cells/mm² SMA+ area, $P < 0.001$; $62.3 \pm 5.7\%$ vs. $31.8 \pm 4.8\%$, $P < 0.001$).

Smooth Muscle Cells Depleted After Castration Are Replenished by Preexisting Smooth Muscle Cells. Based on our *Gli1*-GIFM analysis, *Gli1* is preferentially expressed in stromal stem/progenitor cells that expand SMCs during regeneration. Because there are three distinct ductal stromal subtypes, we tested whether the generation of new SMCs during regeneration is because of expansion of *Sma*-expressing cells or one of the other two subtypes acting as multipotent stem cells. An *Sma-CreER* BAC transgenic line (36) that specifically labels SMCs was used in GIFM studies. We reasoned that, if SMCs (SMA+) are generated from non-SMCs (SMA-), namely subepithelial cells or wrapping cells, then the cell population marked by *Sma*-GIFM should be gradually diluted out after multiple rounds of regeneration. If, however, SMCs are generated from preexisting SMCs, then *Sma*-GIFM will result in persistent labeling after multiple rounds of I/R. We first verified whether the *Sma-CreER* line specifically labels SMA+ cells in the prostate. We found that, in *Sma-CreER/+; R26^{tdTomato/+}* mice administered a moderate dose of Tamoxifen, almost all tdTomato+ cells were also positive for SMA, with less than three cells per section of DP being SMA- 5 wk after Tamoxifen administration (Fig. S6). We labeled the majority of smooth muscle by administering a high dose of Tamoxifen to *Sma-CreER/+; R26^{lacZ/+}* mice and subjected the mice to one or six rounds of I/R. Interestingly, the labeling was similar, if not greater, after six rounds of I/R, and it was similar after one round of I/R compared with 5 wk post-Tamoxifen (Fig. 5). Furthermore, the great majority of cells marked by *Sma*-GIFM were SMA+ (Fig. S6). These results indicate that depleted SMCs are mainly replenished by preexisting SMA+ cells during I/R. Because very few SMA- cells were labeled after one round of I/R, SMA+ cells primarily, if not exclusively, give rise to SMCs.

Fig. 4. Prostate SMCs marked by *Gli1*-GIFM expand in the nonproximal regions after I/R. (A–C) FIHC staining of *Gli1^{CreER/+}; R26^{Yfp/Yfp}* DP sections for SMA (green) and YFP (red). Representative pictures showing (A) YFP+ Sub, (B) SMC, and (C) Wrap marked by *Gli1*-GIFM. (D and F) Quantification of the number of cells of each YFP+ subtype normalized to the SMA+ area (millimeters squared) in (D) proximal and (F) nonproximal ductal regions in mice after 5 wk non-I/R, mice 14 d after castration (end of I), and after I/Rx1 mice. (E and G) Quantification of each YFP+ subtype as a percentage of the total YFP+ cells in (E) proximal and (G) nonproximal ductal regions after 5 wk non-I/R, at the end of I, and after I/Rx1. Data are presented as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by ANOVA; 4,599 YFP+ cells were counted from three mice. Each data point represents one animal.

Fig. 5. Smooth muscle cells marked by *Sma*-GIFM persist after six rounds of I/R. X-GAL staining of DP sections of *Sma*^{CreER/+}; *R26*^{lacZ/+} mice (A) administered corn oil (control) or after 5 wk (B) non-I/R, (C) I/Rx1, or (D) I/Rx6, Tamoxifen. (Scale bar: 100 μ m.)

Discussion

By using powerful mouse genetics tools, we have characterized key components of the canonical SHH signaling pathway at single-cell resolution in the adult mouse prostate and shown that *Gli1* marks stromal stem/progenitor cells that have long-term self-renewal capacity and repopulate three ductal stromal subtypes after many rounds of I/R. Moreover, using *in vivo* GIFM, we found that HH-responding cells do not expand the epithelium during regeneration, suggesting that high-level HH signaling does not regulate epithelial stem cells in the adult mouse prostate during homeostasis. Furthermore, SMCs are mainly regenerated by preexisting SMA+ cells. By extrapolation and based on the spatial separation of stromal subtypes, we propose a model where each stromal subtype is primarily maintained by its own unipotent stem/progenitor cells, which are responsive to SHH signaling during homeostasis in the adult prostate (Fig. 6).

We identified four stromal subtypes in the adult prostate based on their marker protein profile and position. Interestingly, *Gli1* expression is enriched in subepithelial stromal cells. This finding might reflect the fact that subepithelial cells are closest to a source of SHH (basal cells). During prostate development in the mouse, a thin layer of connective tissue (termed lamina propria) is located between the uroepithelium and muscularis mucosa (37). Additional studies are needed to decipher whether subepithelial cells are derived from cells in the lamina propria, because they have a similar location. Furthermore, we found that each stromal subtype is likely replenished by its own unipotent stem/progenitor cells, a finding that parallels what has recently been shown for the two major epithelial subtypes of the prostate (9–11). Moreover, SMCs marked by *Gli1*-GIFM are preferentially depleted during involution and expand during regeneration. Interestingly, this finding was observed only in the nonproximal regions of the ducts. Although one explanation could be that a higher cell turnover is detected in the nonproximal regions than the proximal regions, another possibility is that cells migrate from proximal to distal as they regenerate the prostate, a model previously proposed for epithelial stem cells (6). When tools are available in the future to differentially fate map proximal versus distal cells, such studies will shed light on whether there is a cellular hierarchy along the proximal–distal axis.

We found that, in the adult prostate, *Gli1*, *Gli2*, and *Gli3* are expressed by stromal cells. Interestingly, *Shh* and *Gli3* are coexpressed in the basal cells, but because *Gli1* is not expressed by these cells, basal cells seem to either not be permissive to HH signaling or undergo low-level signaling that is not sufficient to activate *Gli1*. Therefore, GLI3 likely serves as a transcriptional

repressor in basal cells. It is possible that, under certain experimental or disease conditions, an increase in SHH ligand leads to a decrease in GLI3 repressor and/or production of a GLI3 activator (18), which could result in expression of genes that are normally suppressed by GLI3 repressor. Consistent with the idea that increased SHH could result in prostate cancer, overexpression of SHH ligand by electroporation of the adult mouse prostate was found to result in prostate cancer in 90 d (38). In contrast, overexpression of a constitutively active form of SMO in the postnatal prostate epithelium does not lead to increased proliferation or cancer after 12 mo (39). One possible explanation for these seemingly opposite conclusions is that, because overexpression of SHH ligand could affect both the stroma and the epithelium, a deregulation of HH signaling in both compartments is required to initiate prostate cancer.

Stromal cells play a critical role in altering many epithelial disease states. In the prostate, ablation of the TGF- β type II receptor in a subset of fibroblasts can promote epithelial tumorigenesis (40–42). Also, overexpression of FGF10 in embryonic urogenital sinus mesenchyme promotes cancer in adult dissociated prostate epithelial cells grafted under the kidney capsule (43). Furthermore, in human prostate cancer, the extent of reactive stroma correlates with disease severity and can be used as a prognostic indicator for disease-free survival (44). As well, prostate stromal stem cells have been proposed to contribute to the pathogenesis of BPH in some instances (45). In light of our finding that SHH signals to unipotent stromal stem/progenitor cells, it is important to explore whether increasing HH signaling in prostate stromal stem cells leads to overgrowth of the stroma and whether paracrine signaling then alters the epithelium. In this regard, identification of HH target genes in prostate stroma would then help elucidate how abnormal stromal–epithelial interactions can contribute to BPH and prostate cancer, which is currently incurable at its advanced stage.

Materials and Methods

Mice. The following mouse lines were used: *Shh*^{lacZ/+} (29), *Gli1*^{lacZ/+} (17), *Gli2*^{lacZ/+} (31), *Gli3*^{lacZ/+} (4), *Gli1*^{CreER/+} (2), *Sma*-*CreER/+* (36), and *Rosa26* (R26) reporter mice (46–48). To induce genetic recombination, 20 mg/mL Tamoxifen (T5648; Sigma) dissolved in corn oil were given to mice by oral gavage (the doses of Tamoxifen were as follows: *Gli1*^{CreER/+}; *R26*^{lacZ/+}, *Sma*^{CreER/+}; *R26*^{lacZ/+}: 250 μ g/g; *R26*^{CreER/lacZ}, *Gli1*^{CreER/+}; *R26*^{Yfp/Yfp}: 125 μ g/g; *Sma*^{CreER/+}; *R26*^{tdTomato/+}: 62.5 μ g/g). Mice that had undergone 12 rounds of I/R were given three doses of 250 μ g/g Tamoxifen. Non-I/R mice were killed 5 d (5 d non-I/R) or 5 wk (5 wk non-I/R) after Tamoxifen treatment. I/R mice were castrated 7 d after the administration of Tamoxifen, and androgen pellets (5 mg; Innovative Research of America) were implanted s.c. 2 wk after

Fig. 6. Summary of SHH signaling in the adult prostate and model of stromal regeneration. Schematic showing that *Shh* and *Gli3* are coexpressed in the basal cells (BCs), that *Gli1* expression is enriched in Subs, and that *Gli1*, *Gli2*, and *Gli3* are expressed in all four stromal subtypes: Subs, SMCs, Wraps, and IFs. Curved arrows indicate our proposed model that SHH signals to four lineage-restricted unipotent stromal stem/progenitor cells, three of which are shown. Based on our *Sma*-GIFM results, SMCs are replenished by preexisting SMCs (red arrow). By extrapolation and the spatial separation of the three subtypes, we propose that Subs and Wraps are replenished by distinct stem/progenitor cells (white arrows). BM, basement membrane; LC, luminal cell.

castration. I/R mice were killed 2 wk after androgen replenishment. Mouse husbandry and all experiments were performed in accordance with Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee-approved protocols.

Tissue Processing and Staining. Prostates were harvested and dissected in cold PBS, and they were fixed in 4% (vol/vol) paraformaldehyde at 4 °C for 20 min (for X-GAL) or overnight (for FISHC). Prostates were embedded in frozen optimum cutting temperature (OCT) compound (VWR) and sectioned at 12 μ m. Sections of the dorsal prostates were used for all analysis. For immunostaining, cryosections were stained with the following primary antibodies: bGAL (ab9361; Abcam), CK5 (PRB-160P; Covance), CK8 (TROMA-I; DSHB), EPCAM (14-6773; eBioscience), SMA (F3777; Sigma), CD34 (14-0341; eBioscience), GFP/YFP (A-11122; Invitrogen), calponin (ab46794; Abcam), smooth muscle myosin heavy chain (ab53219; Abcam), and laminin (ab11575; Abcam). Secondary antibodies for double labeling were donkey antispecies conjugated with Alexa Fluor 488 or 555 (Molecular Probes). Nuclei were counterstained with DAPI. The Click-it EdU assay with Alexa Fluor 647 (C10340; Invitrogen) was used according to the manufacturer's protocol. TUNEL staining was carried out according to the Roche Applied Science protocol. X-GAL staining was according to standard protocols. Additional details are in *SI Materials and Methods*.

Quantification. To quantify the area of cells expressing LacZ, 10 $\times$ mosaic photographs of three region-matched dorsal prostate sections were taken from each male, and the X-GAL+ area and total ductal wall area (excluding the interstitial area and lumen) were measured using Stereo Investigator (MBF Bioscience). To quantify the YFP+ or EdU+ cells, 20 $\times$ mosaic photographs of at least three region-matched dorsal prostate sections were taken from each male, and positive cells were counted manually using Stereo Investigator. At least three mice were analyzed for each group in each experiment. Data are presented as mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism version 6.0.

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