Supporting Information

Lawson et al. 10.1073/pnas.0913873107

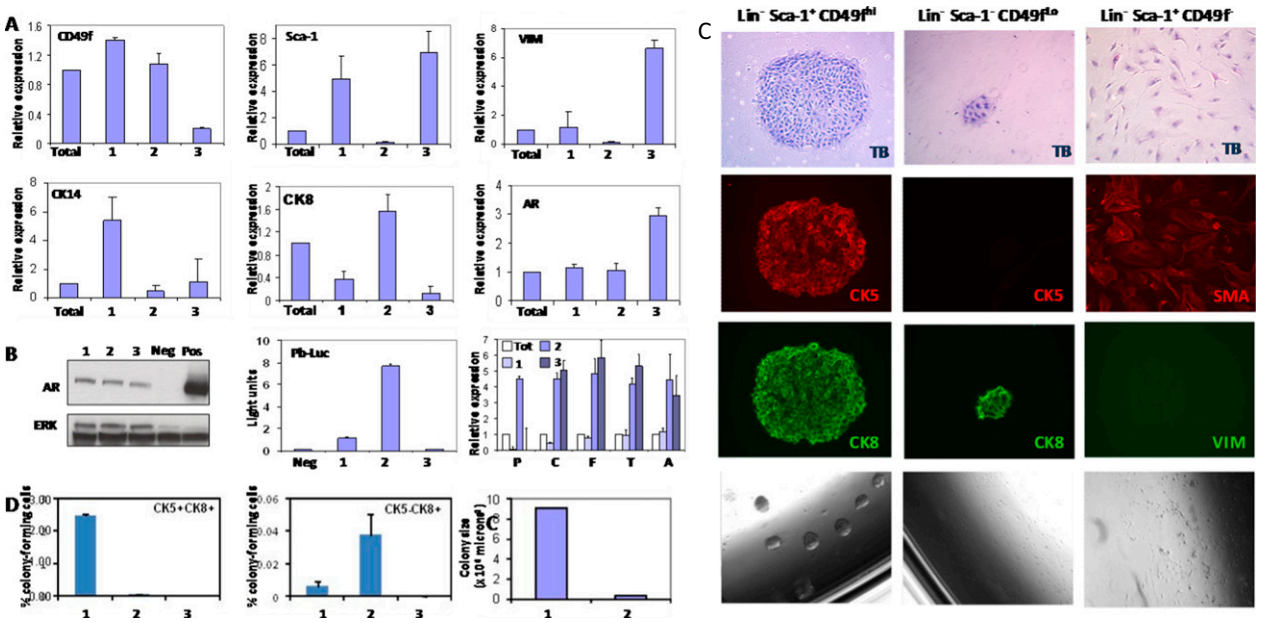

Fig. S1. Basal/stem, luminal, and stromal cells have distinct phenotypic and functional properties. (*A*) qPCR analysis for expression of prostate lineage markers in each population. Expression is relative to total unsorted prostate cells. (*B*) Western blot (*Left*) analysis for androgen receptor (AR) in each population. Neg, negative control, PC3 cell lysate; Pos, positive control, LNCaP cell lysate. (*Center*) Luciferase assay for expression of the enzyme in each population of cells isolated from ARR2 Pb-Lux transgenic mice. (*Right*) The relative expression of a series of androgen-regulated genes in each fraction by qPCR analysis (*P*, probasin; C, calreticulin; F, FKBP51; T, TMEFF2; A, aquaporin 5). Data are shown as expression level relative to total unsorted prostate cells. (*C*) Prostate cells were sorted into each fraction and plated in the in vitro colony- and sphere-forming assays. Staining with trypan blue (TB) and antibodies against CK5, CK8, Vimentin (VIM), and SMA on representative colonies from each fraction are shown. *Bottom* panels show overview images of spheres on Day 10 of culture. (Original magnification: 100×.) (*D*) Serial dilutions of prostate cells (1,000–50,000) from each fraction were plated in the colony assay and the number of each type of colony was counted 8 days later. Graphs show the efficiency of each population for forming primitive CK5+CK8+ (*Left*) and mature CK5-CK8+ (*Center*) colonies. The *Right* graph shows the average area of CK5+CK8+ and CK5-CK8+ colonies.

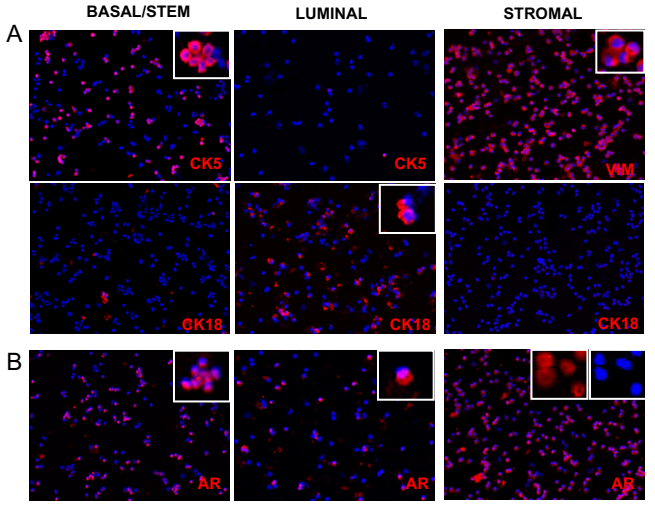

Fig. S2. Immunocytochemical analysis of prostate lineage marker expression in basal/stem, luminal, and stromal cell fractions. (*A*) Cytospins of sorted prostate basal/stem, luminal, and stromal cell fractions were stained with antibodies against CK5, CK18, and VIM (Original magnification: 10×.) *Insets* show higher power view of individual cells. (*B*) Cytospins of each fraction were stained with antibody against *A*. (Original magnification, 10×.) *Insets* show higher power view for examination of nuclear versus cytosplasmic expression of the receptor in individual cells.

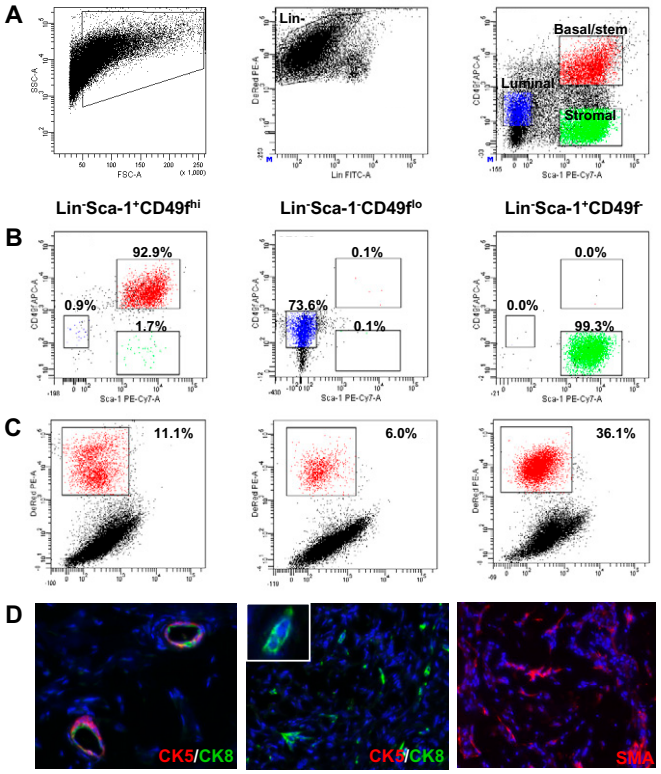

Fig. S3. In vivo differentiation properties of basal/stem, luminal, and stromal cell fractions. (*A*) FACS plots showing forward vs. side scatter (*Left*), lineage marker expression (*Center*), and Sca-1 and CD49f expression (*Right*) in primary prostate cells. (*B*) Postsort purity analysis performed on sorted fractions of Lin$^-$Sca-1$^+$CD49^{fhi}, Lin$^-$Sca-1$^-$CD49^{flo}, and Lin$^-$Sca-1$^+$CD49f$^-$ cells. (*C*) Prostate cells from b-actin dsRED animals were sorted into each fraction, and several replicates of 100,000 cells were implanted in the prostate regeneration assay. Grafts regenerated from each population were harvested 3 weeks later, digested, and analyzed by flow cytometry for dsRED+ cells. (*D*) Immunohistochemical analysis of tissue sections from each graft harvested in *C*. (Original magnification; 200×.) *Inset* (*Center*) shows high power image of a cluster of CK5-CK8+ cells.

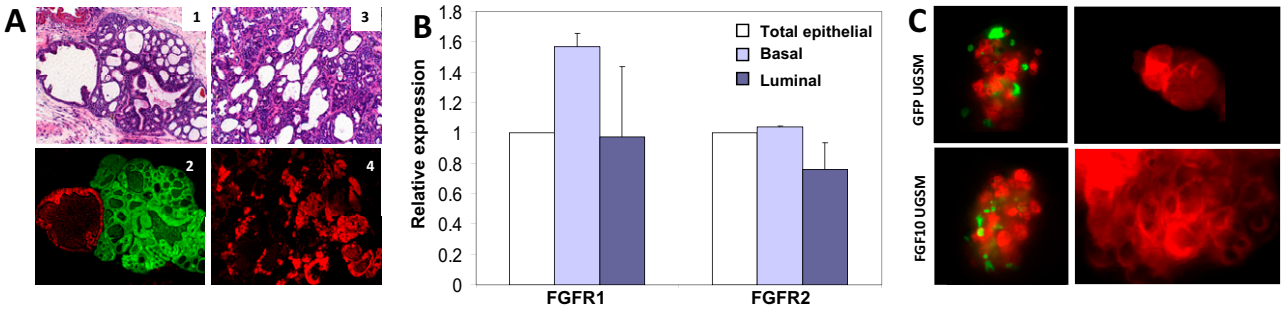

Fig. S4. FGF10 induces increased prostate ductal branching. (*A*) Prostate cells from b-actin dsRED and b-actin GFP mice were sorted by FACS to gate for single cells (excluding doublets and cell clusters), combined together in equal numbers (50,000 each), mixed with GFP- or FGF10-urogenital sinus mesenchymal, and implanted in the regeneration assay. (*Left*) Low-power merged images of dsRED and GFP signal taken by a fluorescence dissecting microscope. (*Right*) Higher-power images of representative ducts from each type of graft. (*B*) H&E and fluorescence images for dsRED and GFP signal in tissue sections from grafts. (Original magnification, 100×.) (*C*) qPCR analysis of FGFR1 and FGFR2 in prostate cell fractions. Expression levels are shown relative to the expression in total epithelial cells.

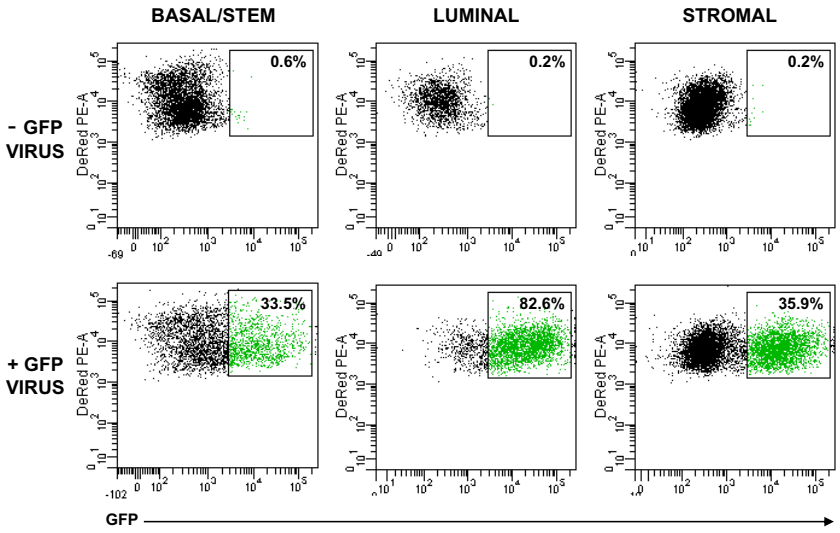

Fig. S5. Basal/stem, luminal, and stromal cells are competent for lentiviral-mediated gene transfer. 100,000 $Lin^{-}Sca\text{-}1^{+}CD49f^{hi}$ (basal/stem), $Lin^{-}Sca\text{-}1^{-}CD49f^{lo}$ (luminal), and $Lin^{-}Sca\text{-}1^{+}CD49f^{-}$ (stromal) cells were mock transduced (– GFP virus) or transduced with GFP lentivirus (+ GFP virus) and implanted in the prostate regeneration assay for 1 week. Harvested grafts were digested and analyzed by flow cytometry for GFP expression.

Table S1. Primer sequences for qPCR experiments

Gene	Sequence
Actin	(5′GATCTGGCACCACACCTTCT3′) and (5′GGGGTGTTGAAGGTCTCAAA3′)
CD49f	(5′ATGGAAGCCCTCAG3′) and (5′CTCTCAACTGCAGC3′)
Sca-1	(5′TCAGAGCAAGGTCT) and (5′ATGGACACTTCTCA3′)
Vimentin	(5′TCAGAGCAAGGTCT) and (5′ATGGACACTTCTCA3′)
Keratin 5	(5′TCAGAGCAAGGTCT) and (5′ATGGACACTTCTCA3′)
Kertain 14	(5′CCTCTGGCTCTCAGTCATCC3′) and (5′GAGCAGCATGTAGCAGCTT3′)
DeltaNp63	(5′GAGAGAGGGCATCAAAGGTG3′) and (5′GGAAAACAATGCCCAGACTC3′)
NKX3.1	(5′CTCCAGAGCCCGACAAAG3′) and (5′CACTTGCTAAGTCCCCTGGA3′)
Keratin 8	(5′ATCGAGATCACCACCTACCG3′) and (5′CTGAAGCCAGGGCTAGTGAG3′)
Keratin 18	(5′AAGGTGAAGCTTGAGGCAGA3′) and (5′CTGCACAGTTTGCATGGAGT3′)
FGFR1	(5′CACTTTGGTCACACGTTGGGTTT3′) and (5′AGATGAAGAGCGGCACCAAGAAGA3′)
FGFR2	(5′AGAAGCGTACGTGGTTGCC3′) and (5′GCTCCTGCTTAAACTCCTTC3′)
Probasin	(5′ATCATCCTTCTGCTCACACTGCATG3′) and (5′ACAGTTGTCCGTGTCCATGATACGC3′)

Table S2. Antibodies used for FACS, IHC, and ICC

Antibodies	Dilution and source
FACS antibodies	
PE anti-CD49f	1:250 (eBioscience)
APC anti-Sca-1	1:1000 (eBioscience)
FITC anti-Ter119	1:250 (eBioscience)
FITC anti-CD45	1:250 (eBioscience)
FITC anti-CD31	1:250 (eBioscience)
IHC and ICC primary antibodies	
Rabbit polyclonal anti-AR	1:200 (Santa Cruz Biotechnology)
Mouse monoclonal anti-p63	1:200 (Santa Cruz Biotechnology, clone 4A4)
Mouse monoclonal anti-cytokeratin 8	1:1000 (Covance, clone 1E8)
Rabbit polyclonal anti-cytokeratin 5	1:1000 (Covance)
Rabbit polyclonal anti-cytokeratin 18	1:200 (Proteintech Group, Inc.)
Chicken anti-vimentin	1:200 (Abcam)
IHC and ICC seconday antibodies	
Alexa Fluor 594 goat anti-rabbit IgG (H+L)	1:1000 (Molecular Probes)
Alexa Fluor 488 goat anti-mouse IgG (H+L)	1:1000 (Molecular Probes)
Biotinylated polyclonal goat anti-mouse	1:250 (DakoCytomation)
Polyclonal goat anti-rabbit	1:250 (DakoCytomation)
Streptavidin-FITC	1:1000 (Invitrogen)
Streptavidin-Alexa594	1:1000 (Invitrogen)

APC, adenomatous polyposis coli; ICC, immunocytochemical; IHC, immunohistochemical.