Supporting Information

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SI Methods

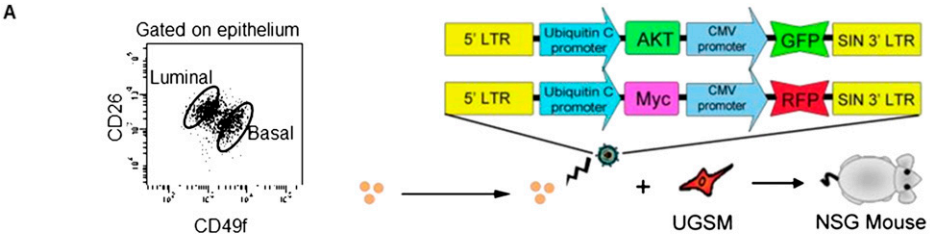
Antibodies. Antibodies used for flow cytometry included CD49f-PE, CD45-APCeFluor 780, HLA-A/B/C-biotin, Streptavidin-APC, and Streptavidin-APC-eFluor 780 (eBiosciences); CD49f-Alexa Fluor 647 and CD26-FITC (BioLegend); and Trop2-APC (R&D Systems). Antibodies used for immunohistochemistry and Western blot included Keratin 8 and Keratin 5 (Covance); Keratin 14 and HLA-A/B/C (Abcam); p63, Erk1/2, and androgen receptor (AR) (Santa Cruz); CD26/DPP4 (LifeSpan Biosciences); chromogranin A (Dako); Myc, eIF4E, and 4EBP1 (Epitomics); Histone H3, AKT, and p4EBP1 (Thr37/46); pErk1/2 (T202/Y204), pSTAT3 (Y705), Sox2, MTA1, Src, and pSrc (Y416) (Cell Signaling); beta-catenin (BD Biosciences); and active beta-catenin (Millipore).

Lentiviral Vectors. The myristoylated AKT vector was previously described (1). For cloning of the Myc vector, the pMX-human-cMYC plasmid was purchased from Addgene (17966), cut with NotI, and blunted using Pfu polymerase. EcoRI linkers (Gene Link) were added to the 3′ end (former NotI site) by overnight blunt-end ligation using T4 DNA ligase (New England Biolabs). EcoRI was used to cut and release the MYC insert. The insert was gel-purified and cloned into the EcoRI sites of FU-CRW lentiviral backbone (2). Insert orientation was checked by ClaI digest and sequenced for confirmation. The resulting plasmid is now described as FU-Myc-CRW with the construct design presented in Fig. S1.

Laser Capture Microdissection, DNA Isolation, and Nonrestrictive Linear Amplification-Mediated PCR. Paraffin-embedded tissues were prepared on PEN membrane slides (Leica) and stained for H&E without a coverslip. Tissue corresponding to squamous and adenocarcinoma regions were isolated using the LMD7000 (Leica). Tissue was collected into a 100-μL nuclease-free PCR tube (Ambion). DNA was isolated and whole genome amplification was performed using the REPLI-g FFPE kit (Qiagen). Amplified human genomic DNA was quantitated against an absolute plasmid standard via probe-based real-time PCR using primers uc483-F (GCATGCTTCATTAACAGTGACC) and uc483-R (TTTAAAATCTGAATGCATGATAAGAATGG) and probe FAM-uc483 (FAM-AGATCCCCAGCTCATCCGTGATTG-Iowa Black) (3). An estimated 100–5,000 genomic equivalents of amplified DNA was used to perform nonrestrictive linear amplification PCR (4). Briefly, 100 cycles of linear amplification were performed with primer HIV3-linear (Biotin-AGTAGTGTGTGCCCGTCTGT). Linear reactions were purified using 1.5 volumes of AMPure XP beads (Beckman Genomics) and captured onto M-280 Streptavidin Dynabeads (Invitrogen Dynal). Captured ssDNA was ligated to read 2 linker (Phos-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3C Spacer) using CircLigase II (Epicentre) in a 10 μL reaction at 65° for 2 h. PCR was performed on these beads using primer HIV3right (AATGATACGGCGACCACCGAGATCTACACTG-ATCCCTCAGACCCTTTTAGTC) and an appropriate indexed reverse primer (CAAGCAGAAGACGGCATACGAGAT-index-GTGACTGGAGTTCAGACGTGT). PCR products were mixed and quantified by probe-based quantitative PCR and appropriate amounts were used to load Illumina v3 flow cells. Paired-end 50bp sequencing was performed on an IlluminaHiSEq. 2000 instrument using a custom read 1 primer (CCCTCAGACCCTTTTAGTCA-GTGTGGAAAATCTCTAGCA). Reads were aligned to the hg19 build of the human genome with Bowtie (5) and alignments were condensed and annotated using custom Perl and Python scripts to locate vector integrations. Integration sites were considered present and significant in a sample if they represented at least 1% of total sequence read alignments.

Inhibitors. HLA+ CD49f^{lo} luminal-like cells were isolated from Myc and myrAKT-driven human prostate tumors by FACS. For drug treatment, Dasatinib (20 nM), Rapamycin (20 μM), and PP242 (10 μM or 1 μM) (all from Selleck Chemicals) or DMSO control (Sigma) were used.

1. Xin L, Lawson DA, Witte ON (2005) The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proc Natl Acad Sci USA* 102(19):6942–6947.
2. Memarzadeh S, et al. (2007) Enhanced paracrine FGF10 expression promotes formation of multifocal prostate adenocarcinoma and an increase in epithelial androgen receptor. *Cancer Cell* 12(6):572–585.
3. Cooper AR, et al. (2011) Highly efficient large-scale lentiviral vector concentration by tandem tangential flow filtration. *J Virol Methods* 177(1):1–9.
4. Paruzynski A, et al. (2010) Genome-wide high-throughput integrome analyses by nrLAM-PCR and next-generation sequencing. *Nat Protoc* 5(8):1379–1395.
5. Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10(3):R25.



B

Naïve benign cells transduced with	Phenotype of cell population	Cell dosage (# tumors /# implantations) 100,000	50,000	10,000	1,000
Myc	Basal (CD45- Trop2+ CD49f^{hi}CD26^{-})	5/5			
	Luminal (CD45- Trop2+ CD49f^{lo}CD26^{+})	0/5			
myrAKT	Basal (CD45- Trop2+ CD49f^{hi}CD26^{-})	5/5			
	Luminal (CD45- Trop2+ CD49f^{lo}CD26^{+})	0/5			
Myc + myrAKT	Basal (CD45- Trop2+ CD49f^{hi}CD26^{-})	6/6	5/5	2/4	0/4
	Luminal (CD45- Trop2+ CD49f^{lo}CD26^{+})	0/6	0/5		

*Pooled data representing experiments using benign cells from 6 different individuals

Fig. S1. Schematic of naïve human prostate in vivo transformation. (*A*) CD45-Trop2+ epithelial cells were sorted based on CD49f and CD26 into CD49f^{hi}CD26^{-} basal-enriched and CD49f^{lo}CD26^{+} luminal-enriched subsets, transduced with lentivirus carrying Myc, myristoylated/activated AKT (myrAKT), or both, combined with urogenital sinus mesenchyme cells and transplanted into NSG mice. (*B*) The number of grafts recovered that contain epithelial lesions per number of grafts implanted in recipient mice is indicated. At doses of 100,000 or 50,000 transformed cells, a single graft was implanted from each patient sample representing five or six individuals. At lower cell doses, duplicate grafts were implanted from two individuals.

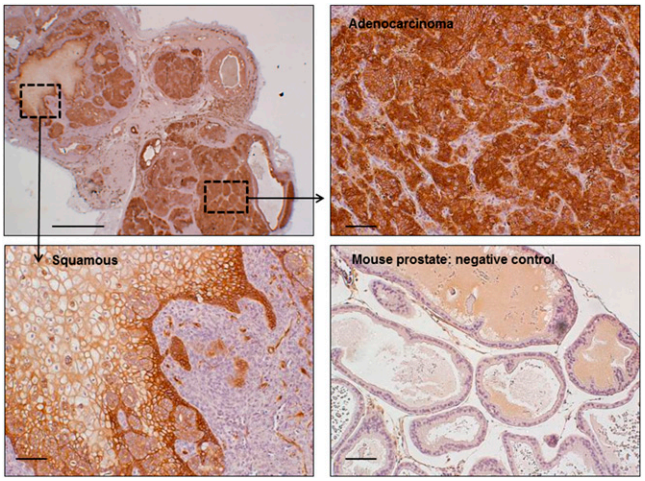


Fig. S2. Species-specific staining for HLA-A/B/C antibody. Tissue sections of human regenerated Myc/AKT tumors and mouse prostate tissue (negative control) were stained with a human-specific pan-HLA antibody to confirm species specificity. (Scale bars, 100 μm; top left overview, 1 mm.)

Tumor replicate	# glands Adeno	# glands Squamous	# glands Mixed	% area Adeno	% area Squamous	% area Mixed
1	1	3	0	65.4	34.6	0.0
2	13	8	0	95.8	9.4	0.0
3	5	12	13	4.0	44.4	51.6
4	15	1	3	74.7	12.3	13.0
5	1	11	5	5.8	84.4	9.9
6	11	11	15	23.4	41.2	35.4
Average	**7.67**	**7.67**	**6**	**44.8**	**37.7**	**18.3**

Fig. S3. Quantification of stand-alone adenocarcinoma, stand-alone squamous, or mixed glands within regenerated tumors. Tissue sections representing Myc- and myrAKT-initiated tumors were subjected to quantification of the number of glands and percentage of total tumor area harboring stand-alone adenocarcinoma, stand-alone squamous, or mixed glands.

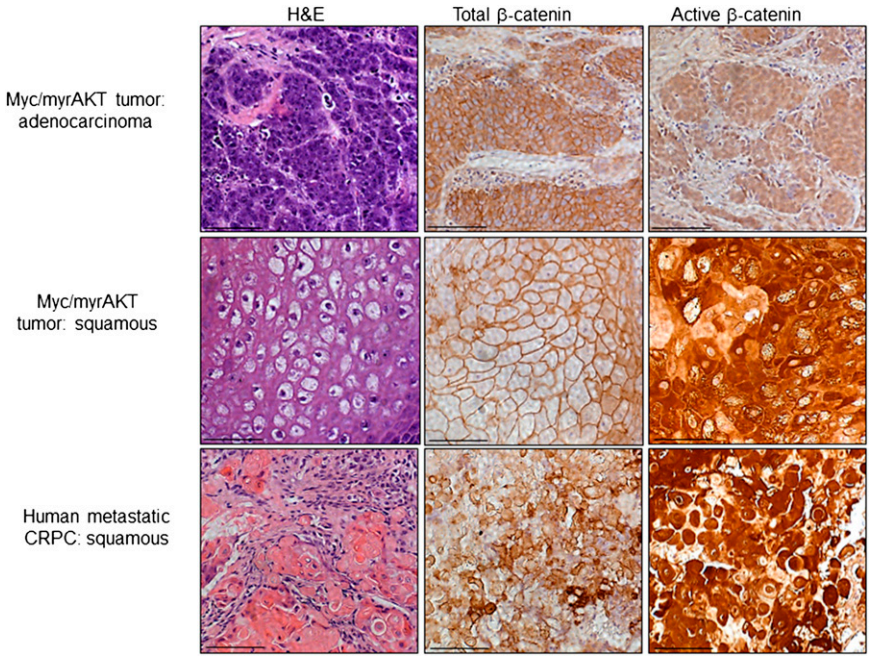


Fig. S4. Elevated expression of active beta-catenin in squamous cells. Representative adenocarcinoma and squamous regions from primary tumors driven by Myc and myrAKT, and a clinical metastatic castration-resistant prostate cancer (CRPC) sample with squamous differentiation were stained for H&E and antibodies against total and activated beta-catenin. (Scale bars, 50 μm.)

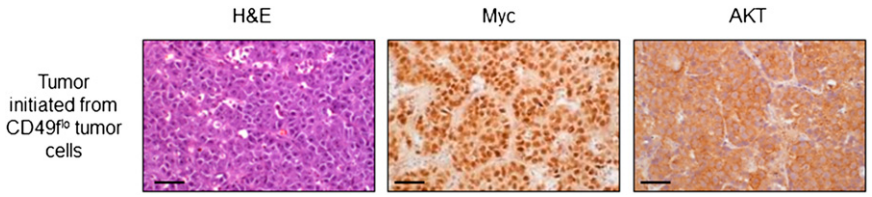


Fig. S5. Characterization of oncogene expression in histological variants present in secondary tumors initiated by CD49f^{lo} tumor cells. Secondary tumors were stained for H&E, Myc, and myrAKT and representative regions are shown. (Scale bars, 50 μm.)

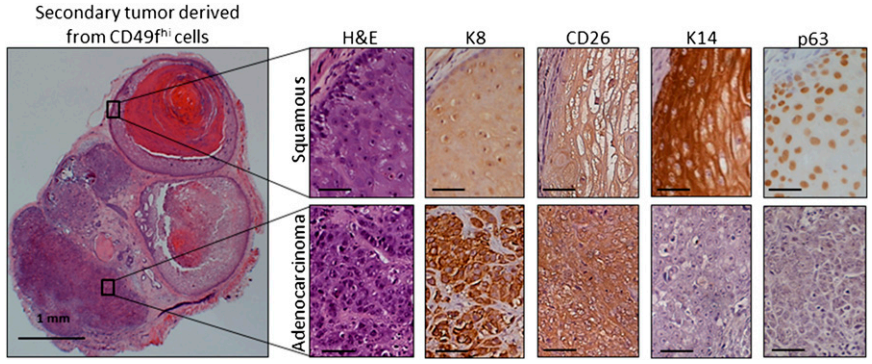


Fig. S6. Tumors initiated from CD49f^{hi} cells expressing Myc and myrAKT were dissociated to single cells, gated based on HLA+, and CD49fhi tumor cells were transplanted back into recipient mice. H&E-stained overview of a representative secondary tumor from 10,000 isolated CD49f^{hi} tumor cells after 6–12 wk in vivo. Both squamous and adenocarcinoma phenotypes are represented in secondary tumors as distinguished by stains for H&E, K8, CD26, K14, and p63. (Scale bars, 50 μm.)

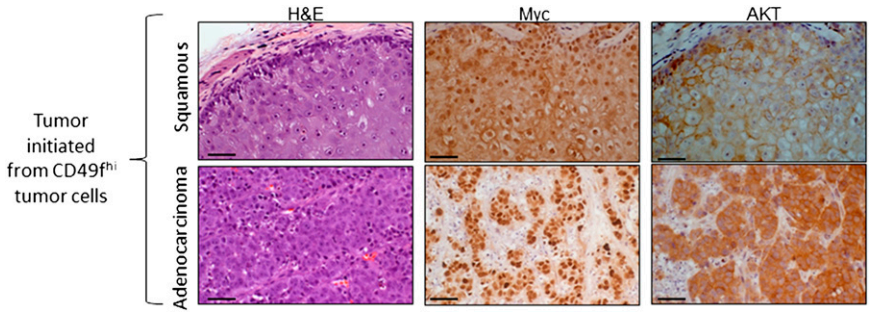


Fig. S7. Characterization of oncogene expression in histological variants present in secondary tumors initiated by CD49f^{hi} tumor cells. Primary tumors initiated in naïve benign CD49f^{hi} cells expressing Myc and myrAKT were dissociated to single cells. Isolated CD49f^{hi} tumor cells were transplanted into recipient mice to establish secondary tumors. Secondary tumors were stained for H&E, Myc, and myrAKT and representative regions are shown. Regardless of the phenotype of secondary tumors, expression of oncogenes Myc and myrAKT was maintained. (Scale bars, 50 μm.)

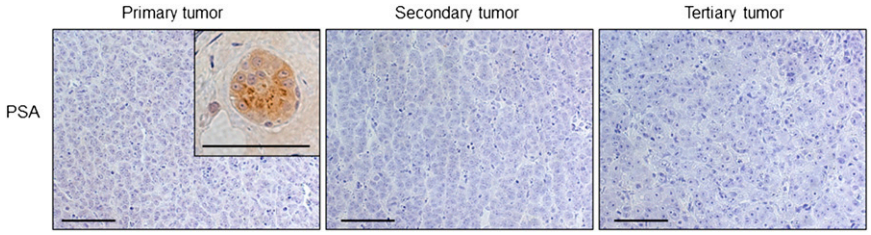


Fig. S8. Low or absent expression of prostate-specific antigen (PSA) in primary, secondary, and tertiary regenerated tumors. Immunohistochemical staining for AR and PSA, a downstream target of AR signaling, in adenocarcinoma regions of regenerated tumors. Primary regenerated tumors, initiated in naïve benign CD49f^{hi} cells, show low or negative expression of PSA except for rare PSA+ glands (*Inset*). PSA expression is absent from secondary/tertiary tumors maintained by CD49f^{lo} tumor cells. (Scale bars, 100 μm.)

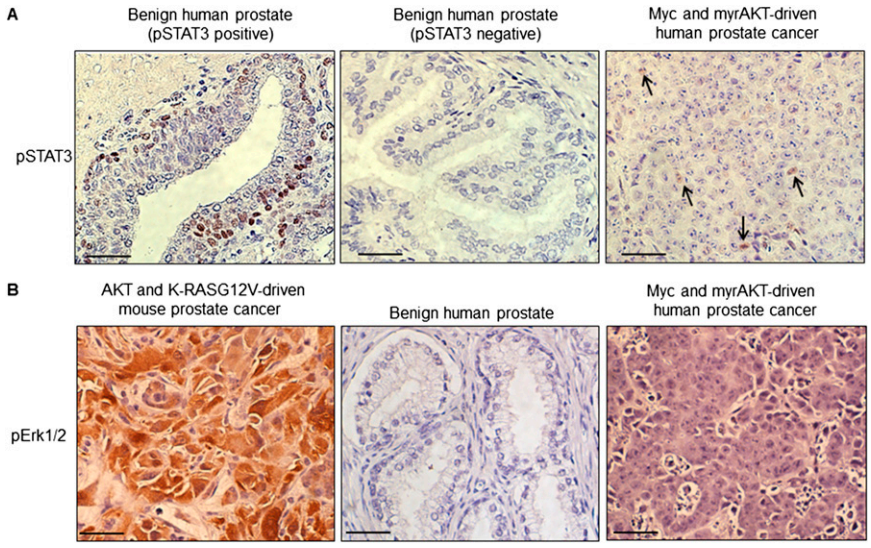


Fig. S9. Myc- and myrAKT-driven human prostate cancer exhibits low or absent expression pSTAT3 and pErk1/2 (*A*) Immunohistochemical staining for pSTAT3^{Y705} in benign human prostate and adenocarcinoma regions of regenerated Myc and myrAKT-driven tumors (arrows denote positive nuclei). (*B*) Immunohistochemical staining for pErk1/2$^{T202/Y204}$ in AKT- and K-RAS^{G12V}-driven mouse prostate cancer (positive control), benign human prostate and adenocarcinoma regions of regenerated Myc and myrAKT-driven tumors. (Scale bars, 50 μm.)

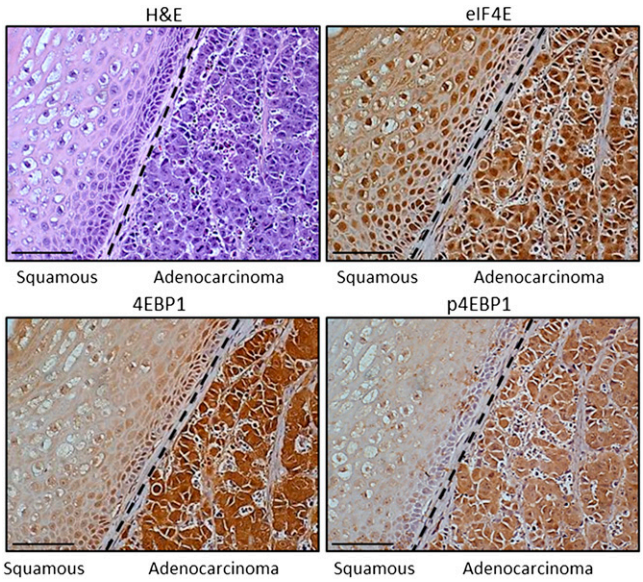


Fig. S10. Expression of EIF4E/4EBP1 pathway components in adenocarcinoma and squamous cells. Immunohistochemical staining for H&E, eIF4E, total 4EBP1, and phosphorylated 4EBP1 (p4EBP1) in Myc/myrAKT-driven primary tumors indicates elevated pathway activation in adenocarcinoma cells compared with neighboring squamous cells. (Scale bars, 100 μm.)