Supplementary Figure Legends

Figure S1

A. IF analysis after GFP-expressing virus injection reveals preference of epithelial infection over stroma. Scale bar 100µm. **B**. IHC analysis reveals normal Pten, pAkt and Ki-67 staining in LV-Luci injected WT (Bl6) prostate. Scale bars, 50µm. **C**. Injection procedures do not trigger an inflammatory response as measured by CD3 staining of prostate tissues or spleen with indicated genotypes. Scale bars, 50µm (middle panel, bottom right), 100µm (all other panels).

Figure S2

Typical example of a RapidCaP experiment visualizing disease progression over time in indicated genotypes.

Figure S3

A. IF analysis of prostates after injection shows specificity of luciferase expression as probed with anti-luciferase antibodies. Scale bars, 42µm (top panel), 15µm (bottom panels).
B. Identification of metastatic prostate cancer nodules in lung using the staining procedure shown in (A). Scale bar, 100µm. **C.** Cytokeratin-8 (Ck8) shows membrane associated staining in normal prostate epithelium, while androgen receptor is nuclear in these cells. In normal lung a similar localization pattern is observed. Scale bar, 100µm. **D**. Prostate cancer metastatic nodules in lung show increased proliferation as measured by Ki-67 staining (compare to Fig. S1B, Ki-67). Scale bar, 50µm. **E**. Nkx3.1 IHC staining is high in normal prostate, present in metastatic lesion and absent from the castration resistant tumor because it inversely correlates with Myc levels (see Ref.). **F**. Myc staining in mock-castrated prostate is weaker than that seen after castration (Fig. 6C, Myc) or in lung metastases (Fig. 7A). Scale bar, 50µm.

Figure S4

Molecular pathology of lung metastasis on three additional cases. Top panels show luciferase signal in post-mortem analysis (scale bar, 1 cm), bottom panels confirm loss of Ck8 in metastatic cells that are also positive for the prostate marker Nkx3.1 and for Myc, but negative for pAkt

(Ser^{473}). Note that lung epithelial stroma serves as internal control for positive pAkt staining (case 3). Scale bar, 100μm.

Figure S5

A. The summary of castration experiments shows degrees of response in primary and secondary disease and exponential tumor relapse as revealed by luciferase imaging and its quantification (small spider plot inset). Note that the imaging data for Cast-1 (third row) and Cast-2 (first row) from Fig. 5 are here presented to show the complete study cohort. Castr., day of castration, low, lowest residual signal, endpt., study endpoint based on morbidity or tumor burden. **B**. Mock-castration shows linear disease progression (left panels) and castration of normal control animals (right panels) confirms negative effect of castration on normal prostate as measured by luciferase (compare also to Fig. 6B). **C**. Summary spider plot for the relative change in tumor burden over time on the entire study cohort. Note that the plot is capped at 120 days post castration follow-up to allow visual comparison of the linear tumor growth (mock castration) with exponential growth (castration).

Figure S6

Summary of castration resistance in RapidCaP. Analysis of six additional cases demonstrate increased prostate size (slide overview column, scale bars, 1cm) and highly consistent histopathology phenotype of CRPCs (H&E column) characterized by sheets of anaplastic tumor cells with pale hematoxilin staining and prominent nucleoli and high nuclear to cytoplasmic volume ratio (asterisks). These cells stain strongly positive for Myc but are for the most part entirely negative for pAkt (Ser^{473}) staining while some retain weak staining. Scale bars: black, 100μm; white, 10μm.

Figure S7

A. IHC analysis of a mixed gland with high pAkt in region 1, and emergence of pAkt-negative cells in regions 2, which are also pS6-deficient. Note that Myc and Ar staining are positive in the glands of both regions 1 and 2, and that Ck8 negative cells in region 2 correlate with loss of pS6 and also with loss of pAkt signal. Scale bar, 50μm. **B**. Map of the retroviral *Myc* luciferase expression vector. **C**. Further examples of local disease dissemination in RV-Myc-luci injected

mice from prostate (yellow arrows) to secondary sites (green arrows), including local lymph nodes. See also Fig. 7C. **D**. Reverse transcript qPCR analysis of *Myc* reveals over-expression in injected prostate at harvest, 416 days p.i., $p = 0.0012$.

Figure S8

A. Live and post-mortem analysis of RapidCaP animals and tissues used for molecular analysis in (**B)** reveals disease spread from prostate to lymph nodes. **B**. RT qPCR analysis of Myc RNA transcription reveals significant reduction in prostate and lymph nodes already after 4 days of treatment with JQ1. Error bars are S.D on the two biological replicates with three experimental replicates, each. $p = 0.05$ and $p = 0.0014$, as indicated.