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Supplementary Figure S1. Copy Number Alteration analysis of human prostate cancer confirms loss of PTEN and TP53 genes as a genetic hallmark of metastatic prostate cancer.

(A) Co-deletion frequency for PTEN and TP53 in metastatic and primary prostate cancer samples. (B) Co-deletion frequency for PTEN and TP53 in metastatic and primary prostate cancer samples. (C) Co-deletion frequency for PTEN and TP53 in metastatic prostate cancer samples. (D) Gene maps of the conditional alleles used for ablation of Pten/Trp53 genes and activation of tdTomato. Cre leads to recombination of loxP sites in the Pten/Trp53 genes and excision of a STOP cassette that leads to activation of the fluorescence protein tdTomato. (E) Analysis of tdTomato-positive cells using Guava flow cytometry system shows that AdCre leads to activation of tdTomato in over 95% percent of cells without the need for selection. (F) Activation of tdTomato can be easily visualized using fluorescence microscopy.

Supplementary Figure S2. Genetic tools used for dissecting Il6/Stat3/Myc signaling.

(A) Scheme of conditional alleles and PCR primers used for assessing the recombination of Pten/Trp53 genes. (B) Senescence-specific β-gal staining is detected after Pten loss but not after Pten/Trp53 co-deletion. Scale bar, 200 µm.

Supplementary Figure S3. Tools used for targeting of Il6 and Stat3.

(A) Il6 neutralizing antibody specifically decreases the proliferation of Pten/Trp53 deficient primary MEFs. ANOVA, Dunnett's post-hoc test, **p<0.01, *p<0.05 vs wt, error bars are SD, n=4. (B) Il6-neutralizing antibody decreases phosphorylation of Stat3 compared to control IgG. Inhibition of Stat3 phosphorylation leads to inhibition of the Stat3 transcriptional target, Myc. (C) Western analysis of Stat3 activation in Pten/Trp53 deficient primary MEFs shows partial response to the pan PI 3-kinase inhibitors LY294002 but not to rapamycin. (D) Guava flow cytometry system analysis of genetic interference with Stat3 signaling by mutant Stat3 plasmid expression.

Supplementary Figure S4. Activation of Stat3/Myc signaling is specific to Ptenpc-/-; Trp53pc-/- prostate and leads to stromal expansion.

Supplementary Figure S4. Activation of Stat3/Myc signaling is specific to Ptenpc-/-; Trp53pc-/- prostate and leads to stromal expansion. (A) H&E analysis of prostates from all studied genotypes reveals massive stromal expansion after co-deletion of Pten and Trp53. IHC analysis confirms stromal pStat3/Myc activation specifically in Ptenpc-/-; Trp53pc-/- prostate. Note that pAkt activation is restricted to gland, consistent with triggering paracrine Il6/Stat3/Myc activation. Scale bar, 100 µm. (B) Pten is present in the stroma of Ptenpc-/-; Trp53pc-/- prostates confirming that the expansion of stroma is not driven by spurious Pten/Trp53 recombination. (C) Proliferation in the Ptenpc-/- glands correlates with Akt activation, in contrast to Ptenpc-/-; Trp53pc-/- glands, where it correlates with Stat3/Myc staining.
Supplementary Figure S5. IHC analysis of wild type lung and primary and metastatic tumors.
(A) Wild type lung does not show increased Myc, pAktS473 or Phlpp2 staining comparing to metastatic lung nodules. Scale bar, 100 µm. (B) Il6 analysis of PC metastasis to lung revealed that metastatic nodules expressed Il6. Staining levels varied between 22.5% and 72.8% positive cells per nodule. (C) No Il6 activation is found in the p53-null setting and the Pten-null glands that were free of hyperplasia/ neoplasia. Pten-null glands with proliferating cells were positive for Il6, consistent with their breaking of the p53 response in parts of the tissue (see the positive gland in the right bottom corner of Ptenpc-/-).

Supplementary Figure S6. Ar expression in primary and metastatic tumors.
(A) IHC analysis of the Ar status in RapidCaP metastatic lesions shows no significant Ar staining (left panel) while most prostate glands show strong nuclear Ar staining. Scale bar, 100 µm (B) The wild type and mutant prostates from Probasin-Cre animals show strong Ar staining, Scale bar, 100 µm.

Supplementary Figure S7. Pathway to Pten/ Trp53 deficient prostate metastasis and therapy resistance.
Pten-loss triggers PIP3 signaling to activate AKT, which prompts activation of the p53/ p21/ p16 tumor suppressors and senescence arrest. Suppression and loss of p53 in this context results in cell proliferation and Il6 secretion. Il6 signals both auto and paracrine to activate Myc via the Jak/Stat pathway. Myc induces the Phlpp2 phosphatase which creates a negative feedback loop by dampening Akt activation. Metastases (and castration-resistant tumors) select for increased Myc expression (and gene amplification), and Akt inactivation, demonstrating that Myc supersedes the need for Akt in lethal prostate cancer.