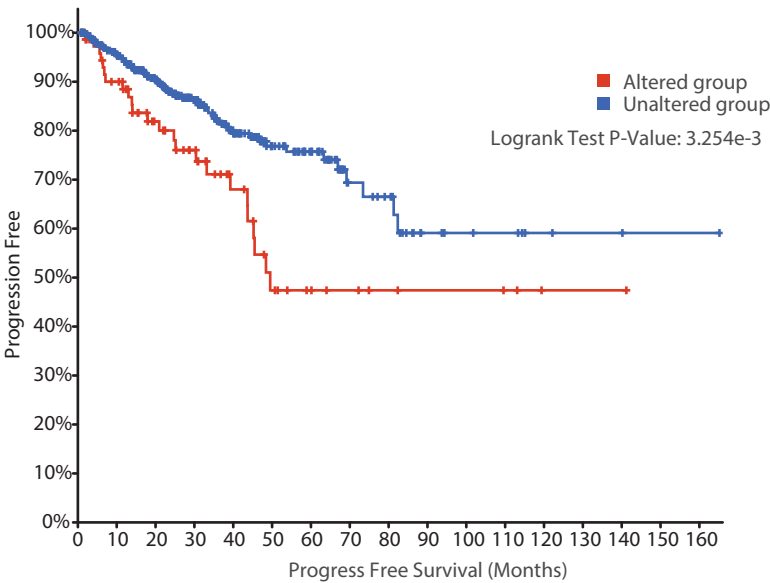
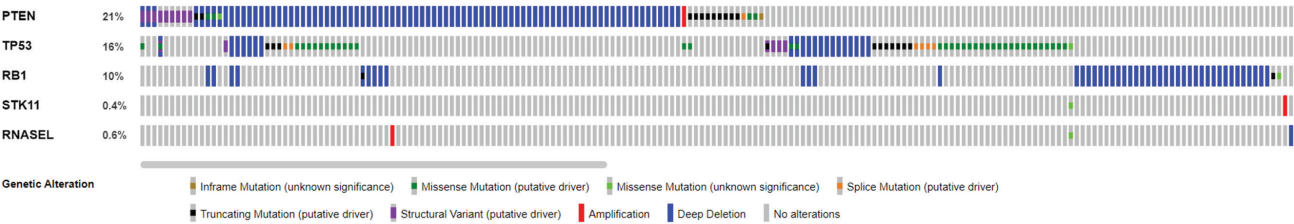


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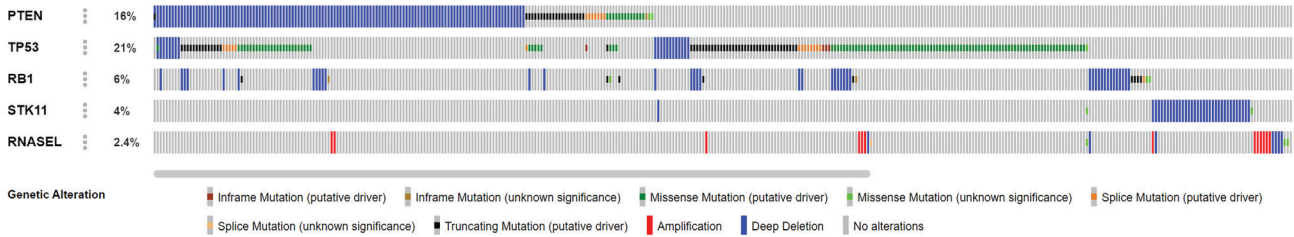


Figure S1: Mutations in TP53, PTEN, RB1, STK11 and RNASEL is associated with worse prognosis

(A) Analysis of progression free survival determined by groups with and without alterations of five tumor repressor genes, TP53, PTEN, RB1, STK11 and RNASEL, based on TCGA_PRAD dataset (n=494). Oncoprint plot showing distribution of mutations in TP53, PTEN, RB1, STK11 and RNASEL in the (B) TCGA_PRAD dataset (n=494), and (C) the MSK/DFCI cohort (n=1013)

A

Gene:	RnaseL	Rb1	Kmt2d	Kmt2c	Zbtb16
indel:	18.90%	55.30%	72.90%	83.30%	91.40%

B

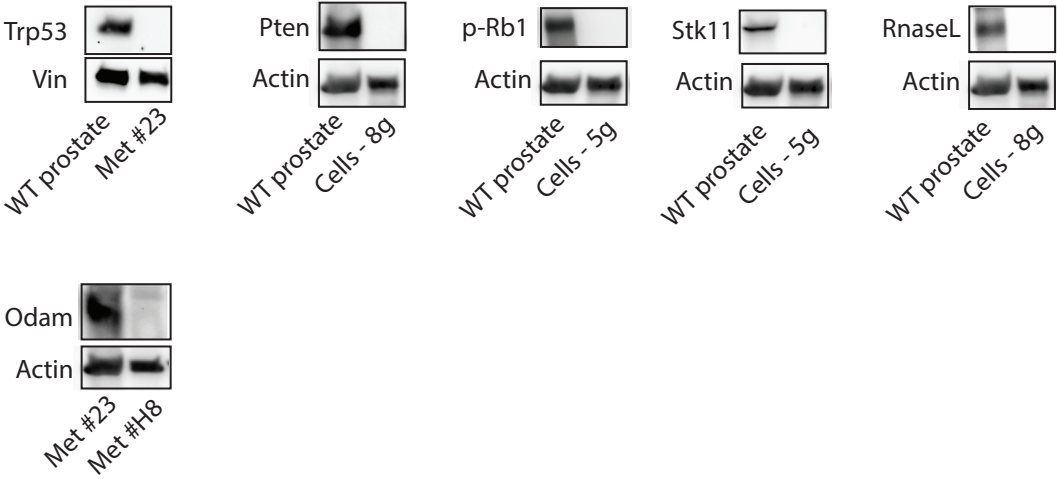
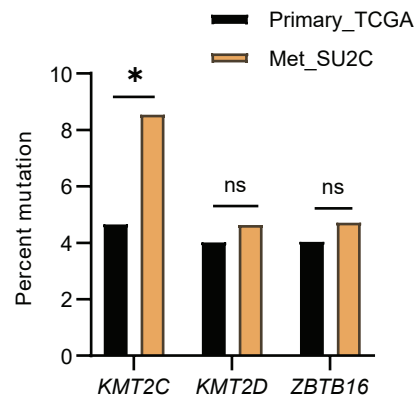


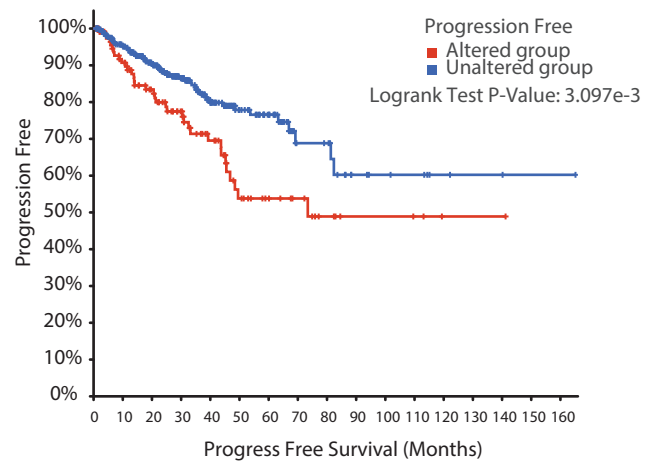
Figure S2: Loss of protein expression by CRISPR induces mutations

A: Induction of indels was validated for novel sgRNAs in MEF cells. B) Cell lines generated from primary or secondary tumors were used to analyze protein expression for genes mutated by CRISPR. Protein from wild-type prostate, or a cell line (Met #23), was used as a control. Vinculin (Vin) or beta-Actin were used as loading controls.

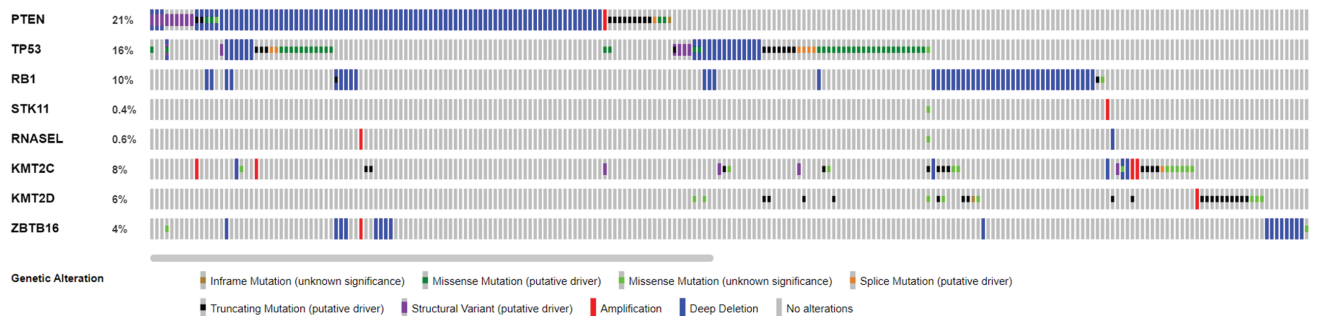
A



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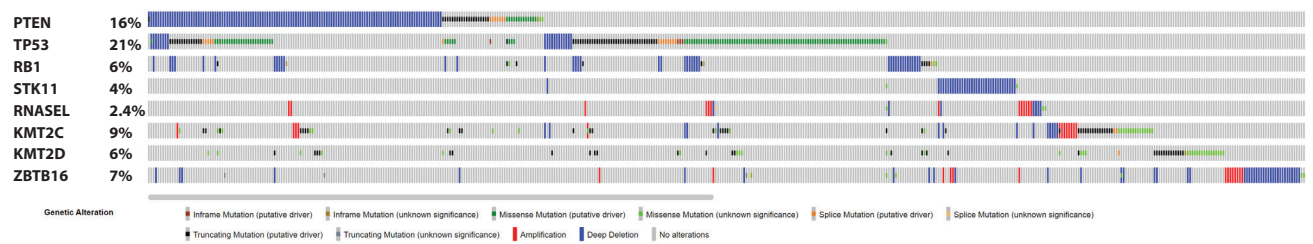


Figure S3: Mutations in KMT2C is increased in metastatic PCa

(A) Percent of mutation counts in KMT2C, KMT2D and ZBTB16 from TCGA-based primary PCa (n=494) and SU2C-derived metastatic PCa datasets (n=429). For KMT2C and D was mutations of unknown significance exclude. Fishers exact test: $* < 0,05$. (B) Analysis of progression free survival determined by groups with and without alterations in eight genes: KMT2C, KMT2D, ZBTB16, TP53, PTEN, RB1, STK11 and RNASEL in the TCGA_PRAD dataset (n=494). Oncoprint plot showing distribution of mutations in the eight genes in (C) the TCGA_PRAD dataset (n=494), and (D) the MSK/DFCI cohort (n=1013)

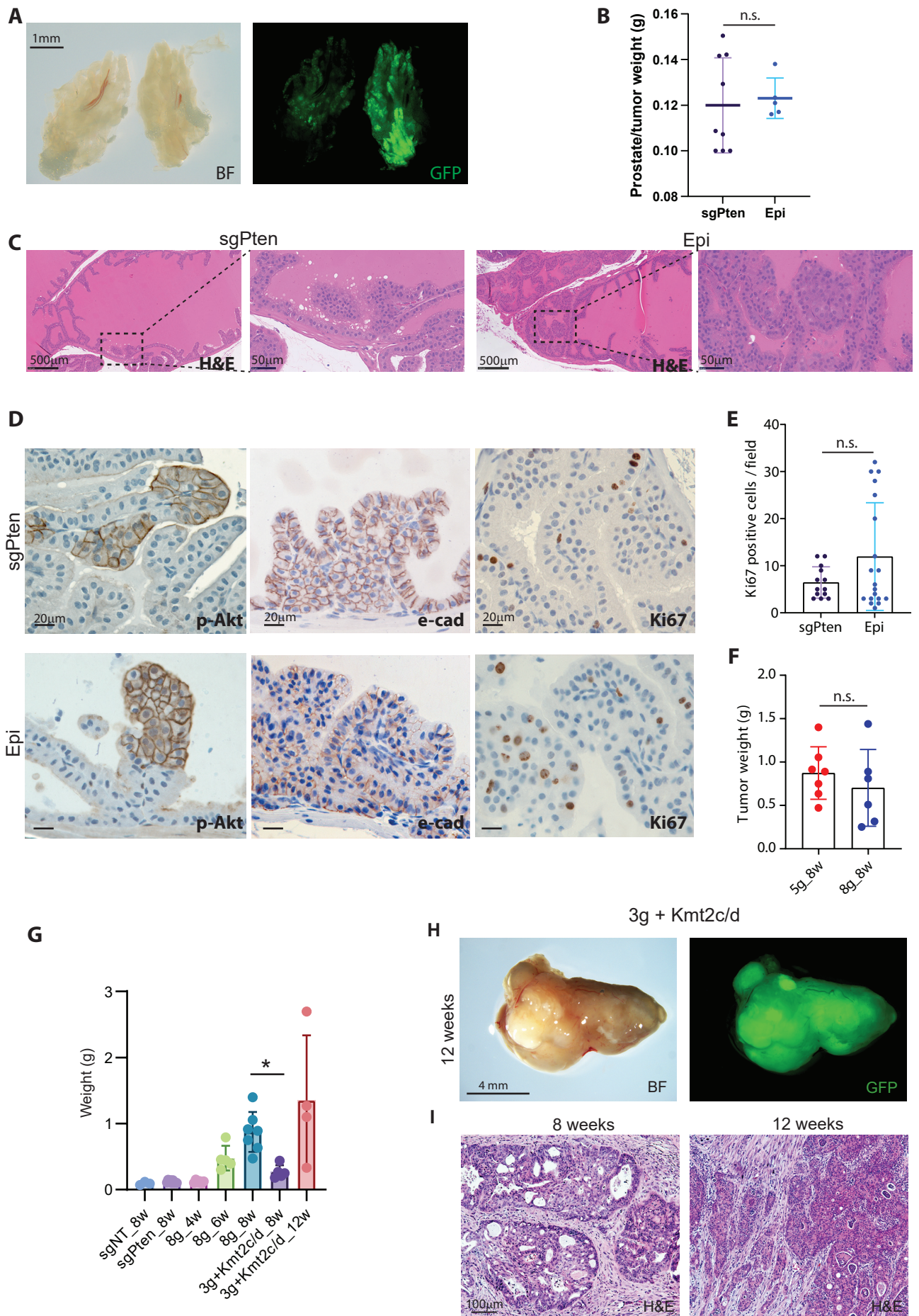
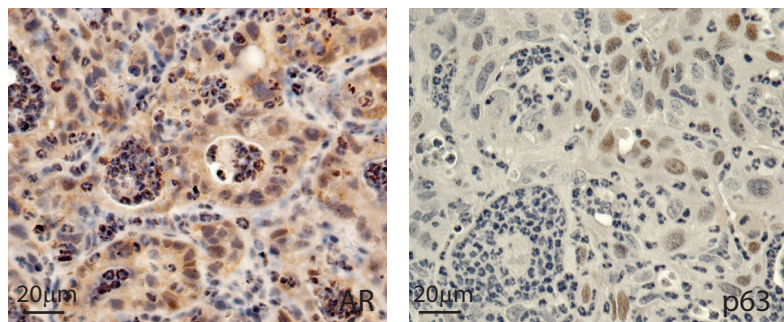


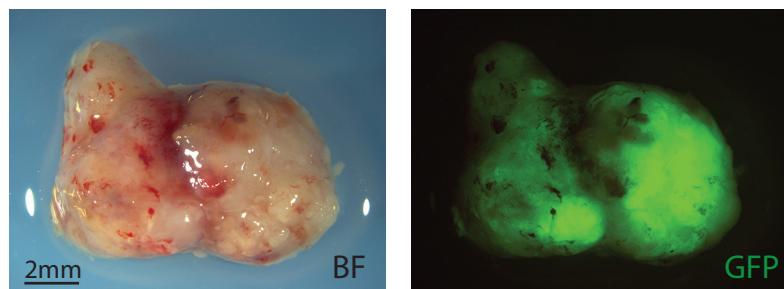
Figure S4: Combinational loss of Pten and three epigenetic factors did not accelerate tumor progression.

(A) Mice were injected with AAV particles targeting Pten and three epigenetic factors, Kmt2c, Kmt2d and Zbtb16. Macroscopic pictures were taken with bright field and GFP channel at 8 weeks after injection. (B) The weight of the prostates from mice injected with Epi-AAV was compared to control mice with only Pten mutated (n=9, 4 for each group). (C) H&E staining indicated the benign prostatic tumors in both sgPten and Epi groups. (D) IHC were performed on tissue sections of sgPten and Epi prostates, to detect abundance of p-Akt, E-cad and Ki67 ($n \geq 3$). (E) Comparison of Ki67-positive cells between sgPten and Epi groups (n=13, 19 fields). n.s.: not significant (Mann-Whitney U test). (F) The weight of the prostates was measured at 8 weeks after administration of the AAV particles for 5g and 8g tumors (n=7). (G) The weight of the prostates was measured at different time points after administration of different AAV particles (*= $p < 0,05$; n=4-7). (H) Mice were injected with AAV particles targeting Pten, Trp53, Rb1, Kmt2c and Kmt2d. Macroscopic pictures were taken with bright field and GFP channel at 12 weeks after injection. (I) H&E staining of paraffin sections from the prostate samples at 8 and 12 weeks after induction. A representative picture is shown (n=4).

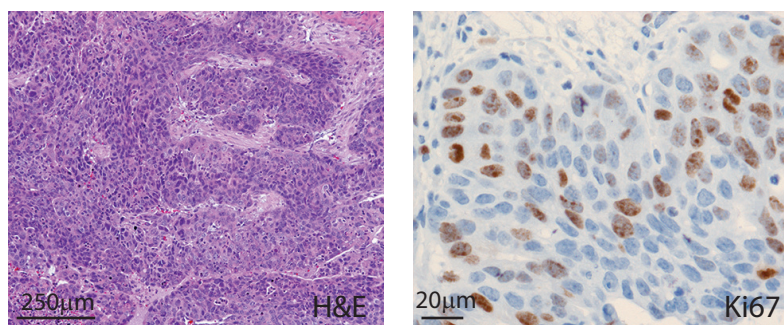
A



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D

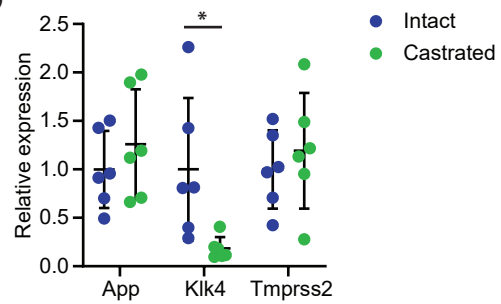
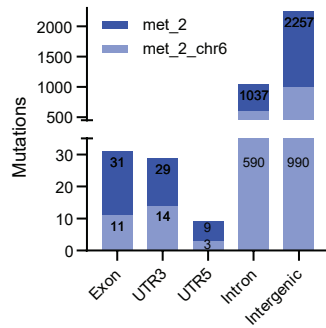
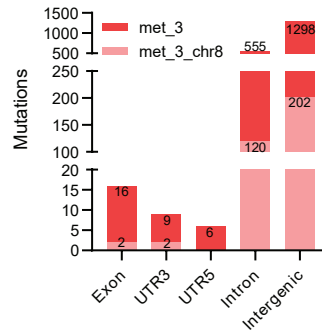


Figure S5: Epithelial derived metastases and castration resistant PCa.

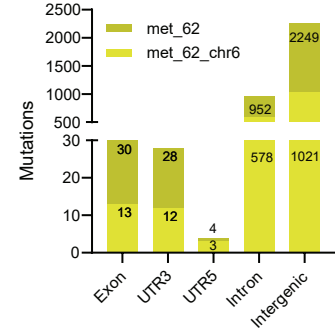
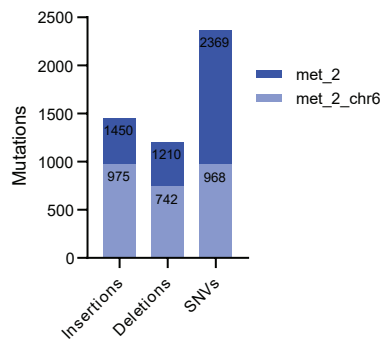
(A) Mice were injected with 8g-derived AAV particles and lung metastases were stained for AR or p63 (n=5). (B) A group of mice were castrated at 5 weeks after injections and sacrificed at 8 weeks. Macroscopic pictures were taken with bright field and GFP channel at 8 weeks and representative pictures are shown (n=7). (C) H&E staining and IHC detecting Ki67 were performed on tissue sections of 8g-castrated mice (n=7). Expression analysis of 3 AR-regulated genes was performed on total RNA from intact or castrated mice at 8 weeks after tumor initiation (n=6, $p < 0.05$).

A Mutations of chr6 in met_2

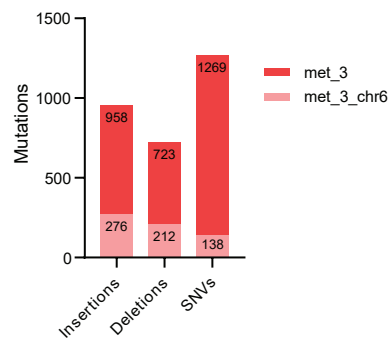
Mutations of chr8 in met_3



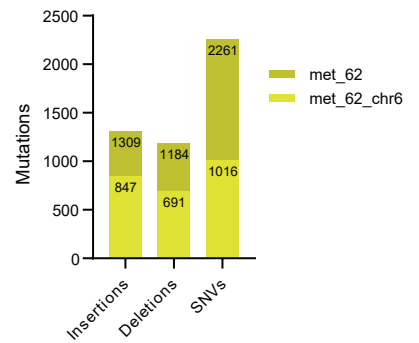
Mutations of chr6 in met_62

**B** SNVs and Indels of chr6 in met_2

SNVs and Indels of chr8 in met_3



SNVs and Indels of chr6 in met_62

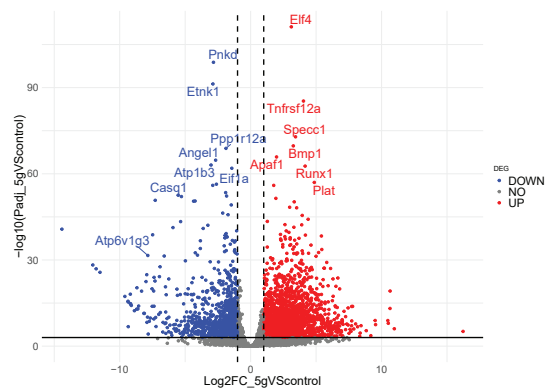
**C**

Genome depth				
Sample	met_2	met_3	met_5	met_62
Average genome	38,7	36,6	37,0	35,6
Average MYC	36,9	36,9	33,9	33,9

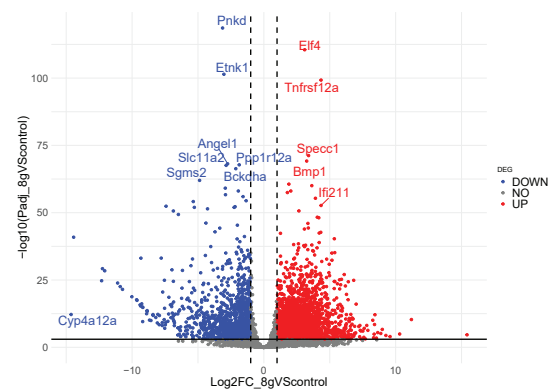
Figure S6: Ectopic and high mutation burden was found at chromosome 6 and 8.

(A) Mutation counts at chr6 for sample lung_2 and Lung_62, and at chr8 for sample lung_3. Genomic features including exon, UTR3, UTR5, intron or intergenic regions are listed. (B) Plots indicating the SNVs, Indels and overall counts at chr6 and chr8 for the representative samples. (C) The average reads for the locus containing c-Myc were compared to the overall read coverage (n=4).

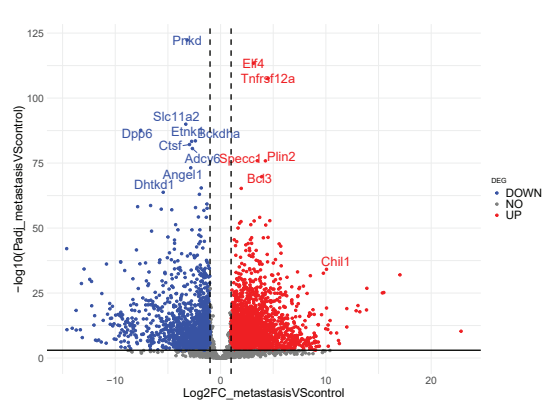
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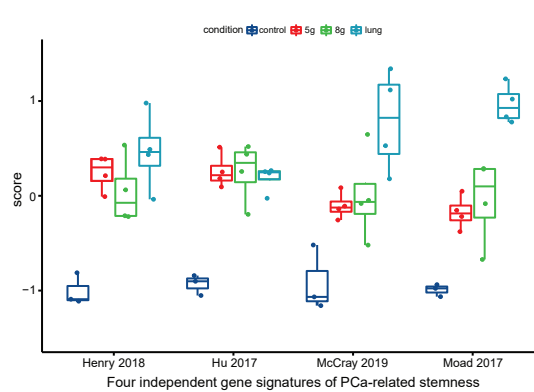


Figure S7: Transcriptome analysis revealed many DEGs in all tumor groups, including PCa-stemness related genes.

(A-C) Volcano plots over DEGs in all tumor groups comparing to control tissues. (D) PCa stemness genes were found to be highly expressed in all tumor samples.

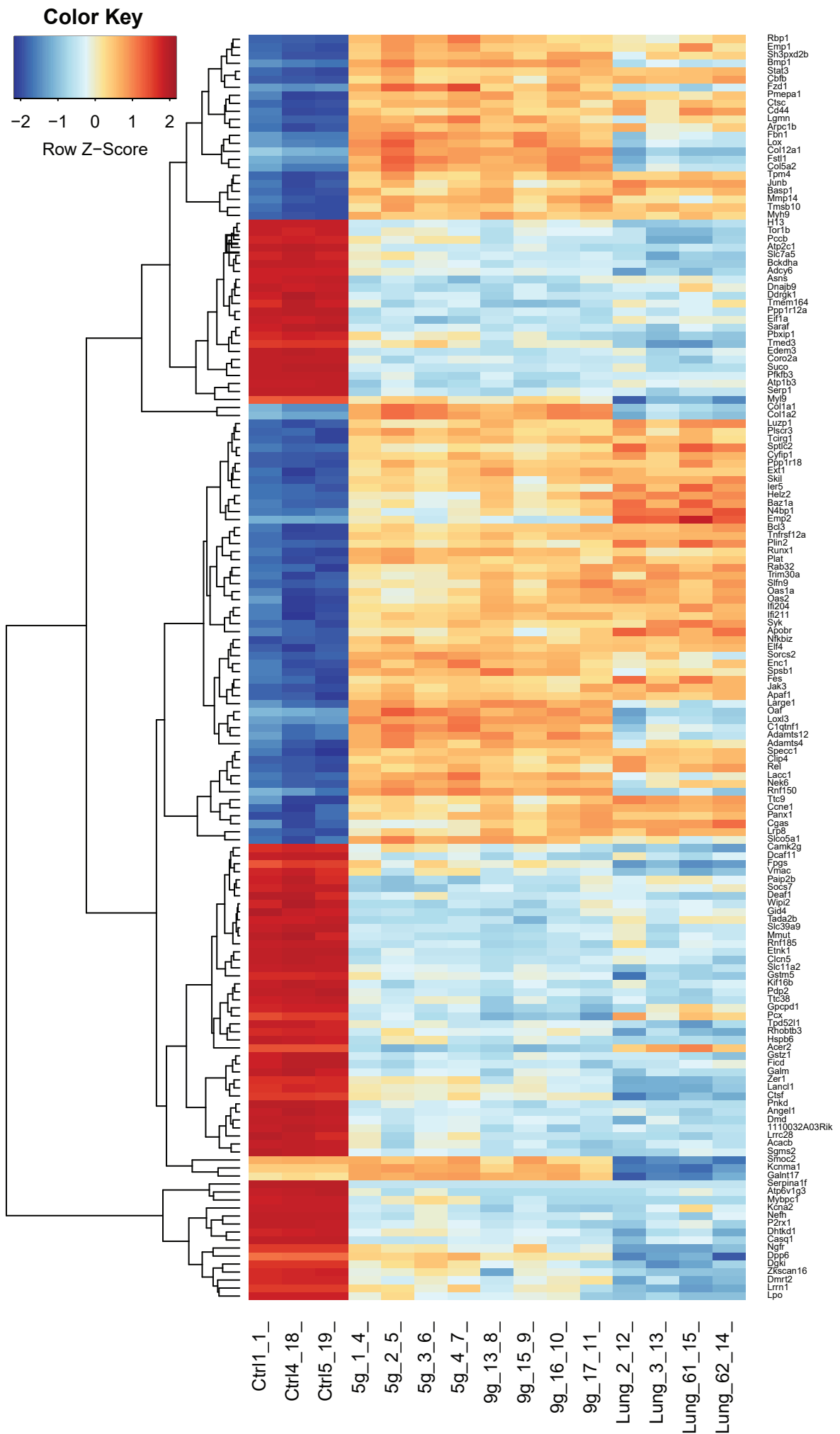


Figure S8: Top dysregulated genes

Heatmap showing the top 100 dysregulated genes between tumor samples and control tissues.

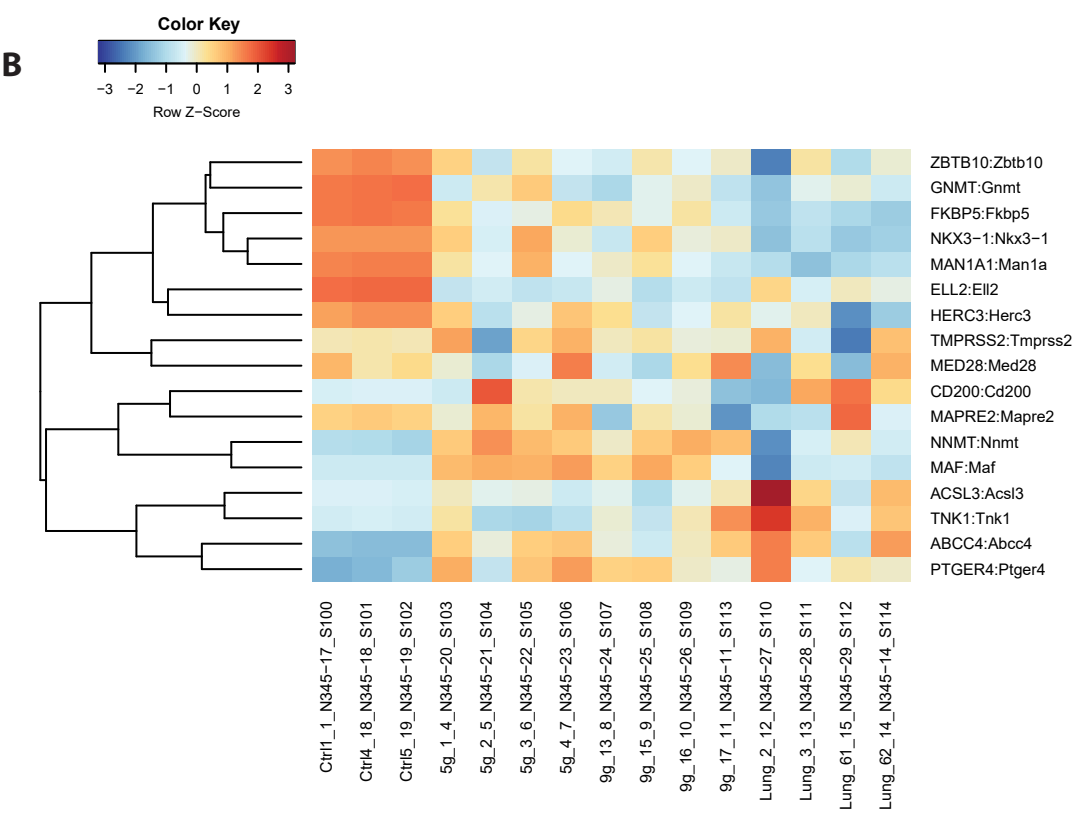
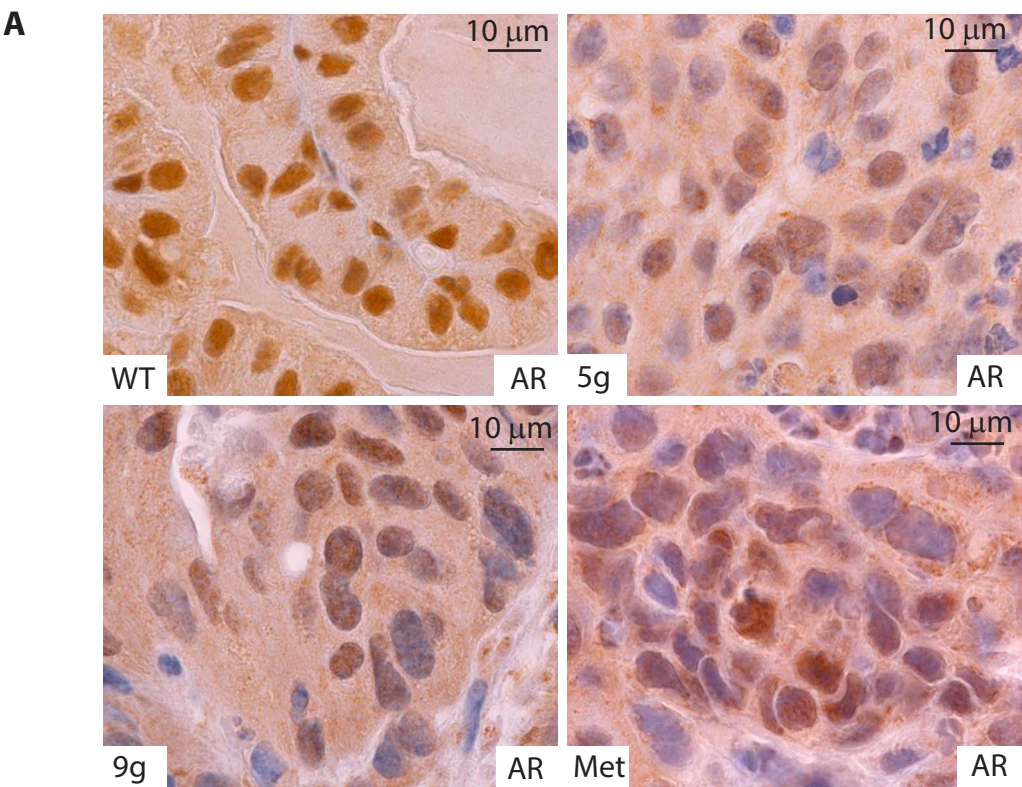


Figure S9: Decreased expression of Androgen-regulated genes

A) IHC staining for androgen receptor (AR) on prostatic tissues from sgPten, 5g and 8g tumors together with lung metastases at 8 weeks after cancer initiation. Representative pictures are shown ($n \geq 5$).

B) Expression of AR-regulated genes was assessed between control tissues and the three tumor groups.

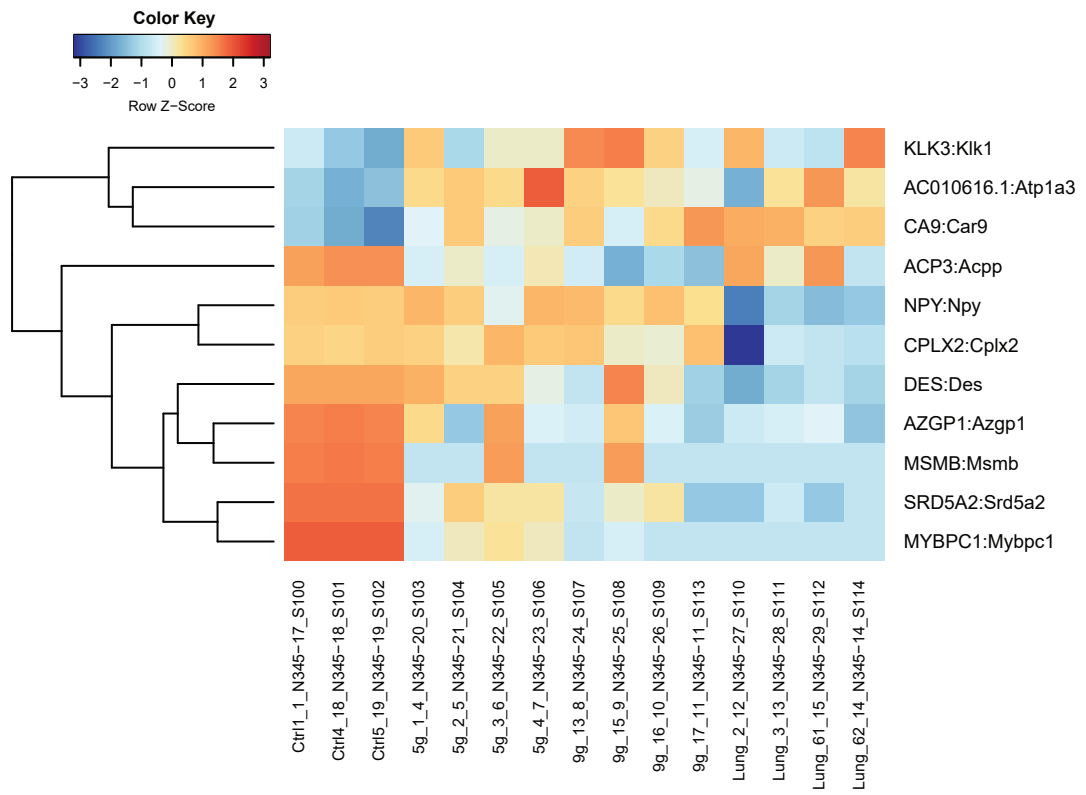
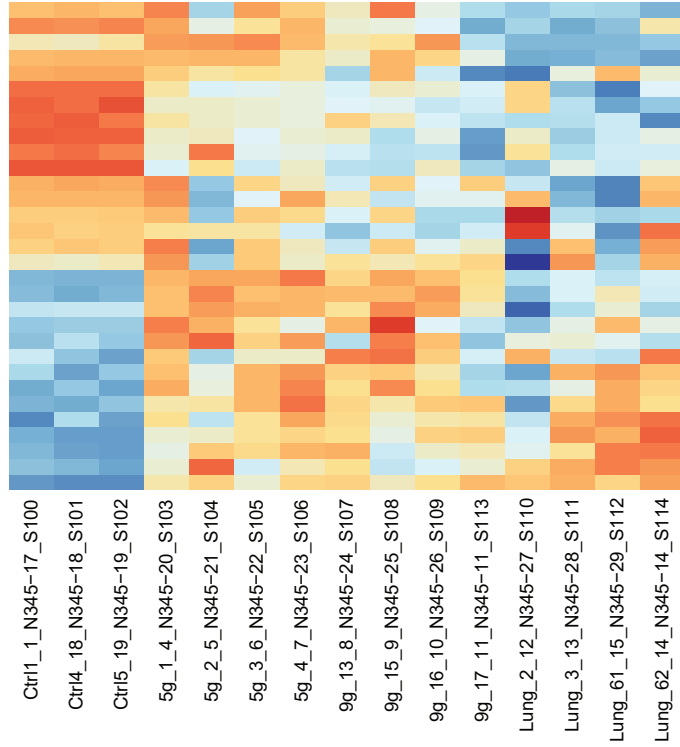
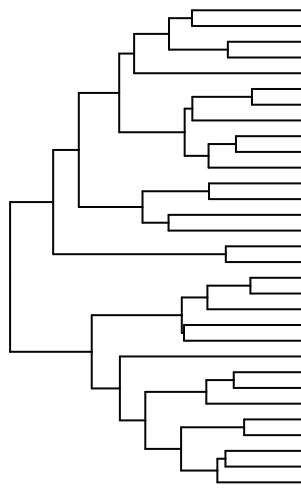
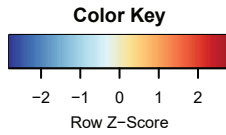
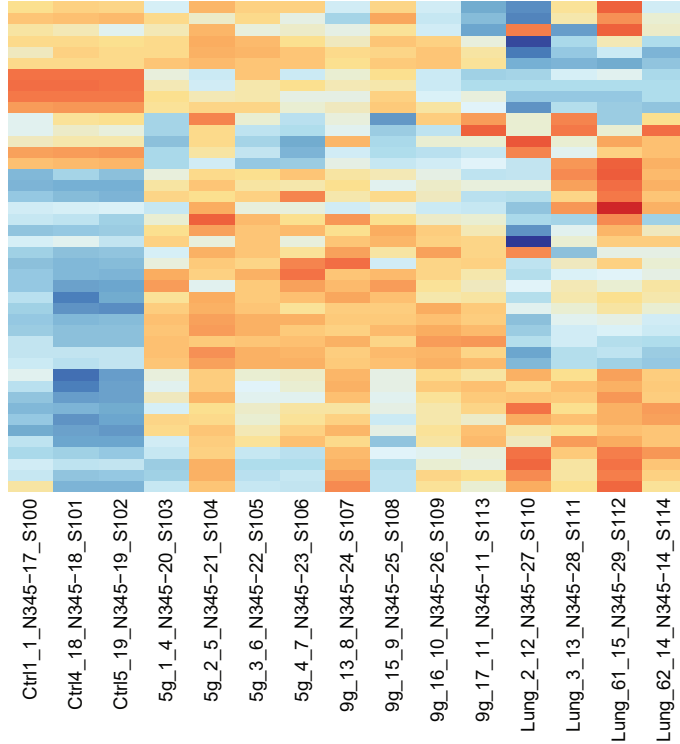
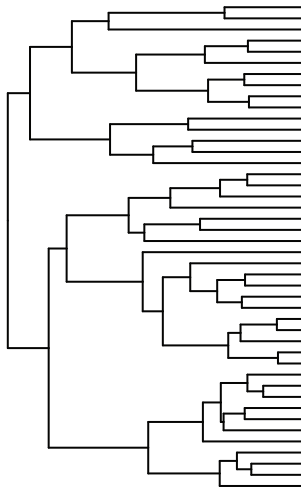
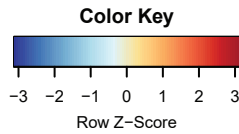


Figure S10: Unchanged neuroendocrine signature

Expression of neuroendocrine related genes was assessed between control tissues and the three tumor groups.

A

FBP1:Fbp1
RAMP1:Ramp1
FOLH1:Folh1
NPTX2:Nptx2
DPP4:Dpp4
AR:Ar
SERHL2:Serhl
ASRGL1:Asrgl1
CWH43:Cwh43
SLC2A12:Slc2a12
TRPV6:Trpv6
SPDEF:Spdef
TSPAN8:Tspan8
UPK1A:Upk1a
PTPRN2:Ptpn2
SYT7:Sy7
TOX3:Tox3
C2:C2
CSGALNACT1:Csgalnact1
GFPT2:Gfpt2
ANKRD1:Ankrd1
PPM1H:Ppm1h
KLK3:Klk1
HLA-DMB:H2-DMb2
TBXAS1:Tbxas1
HLA-DMB:H2-DMb1
LTB:Ltb
DOCK11:Dock11
KRT20:Krt20
DLL4:Dll4
INHBB:Inhbb

B

FHL1:Fhl1
MSRB3:Msrb3
KCNMA1:Kcnma1
FOXI1:Foxi1
TAGLN:Tagln
CDH13:Cdh13
JAG2:Jag2
GIMAP8:Gimap8
ARHGAP25:Arhgap25
NRG1:Nrg1
FJX1:Fjx1
DKK3:Dkk3
LTBP2:Ltp2
TNC:Tnc
SPARC:Sparc
AEBP1:Aebp1
KRT6A:Krt6a
SH2D5:Sh2d5
IL1A:Il1a
WNT7A:Wnt7a
KRT5:Krt5
KRT6A:Krt6b

Figure S11: Altered basal/luminal signature in tumor samples

Expression of basal/luminal genes was assessed between control tissues and the three tumor groups. (A) luminal signature (B) basal signature.

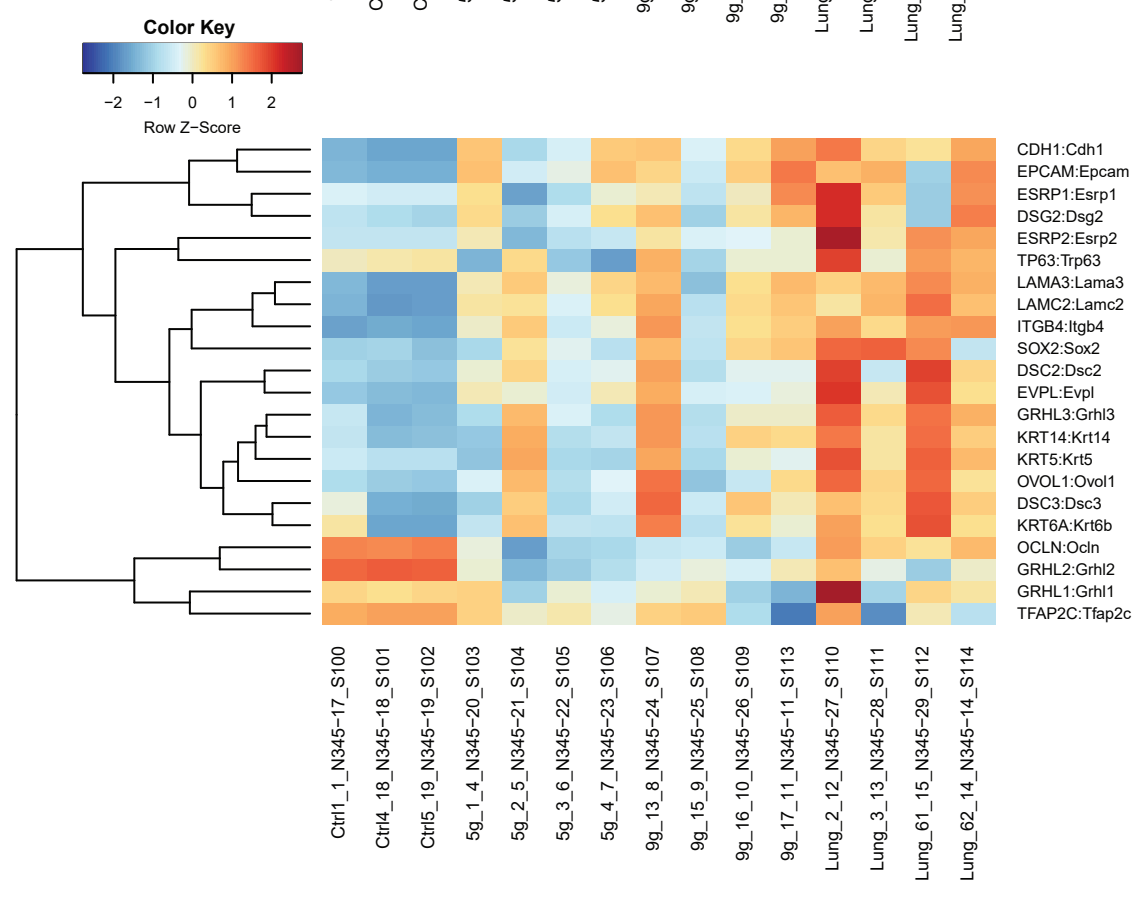
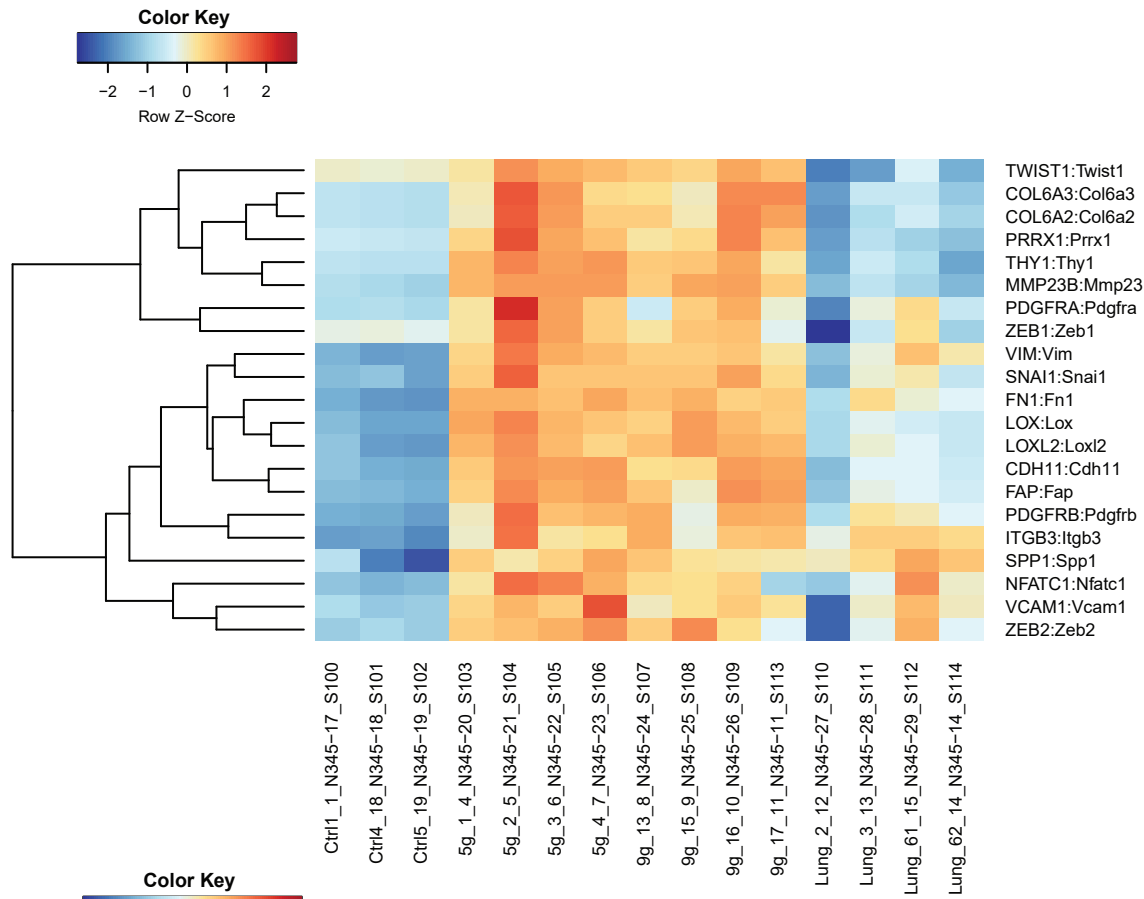


Figure S12: Altered expression of epithelial and mesenchymal signature genes

Expression of epithelial and mesenchymal genes was assessed between control tissues and the three tumor groups. (A) mesenchymal signature (B) epithelial signature.

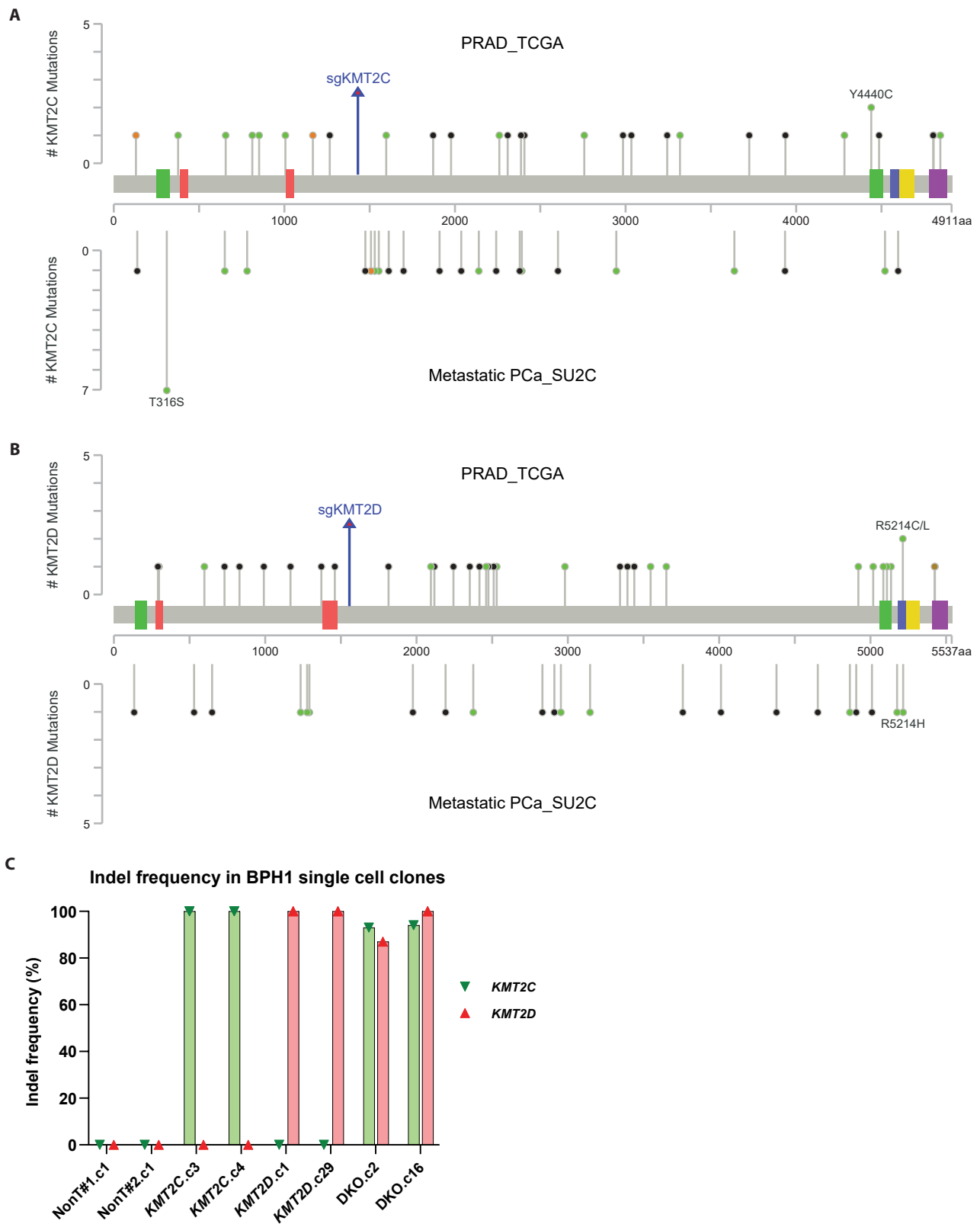
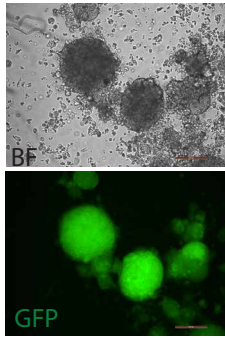


Figure S7

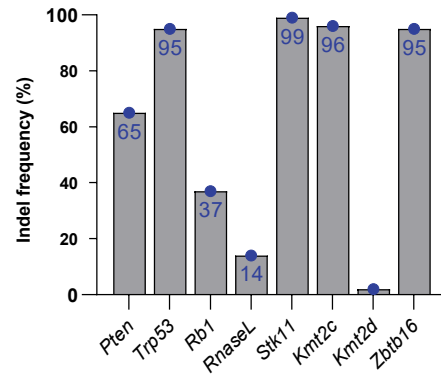
Figure S13: Mutation profile of KMT2C and KMT2D in human datasets and CRIPSR induces mutation to BPH1 cells.

(A, B) Mutation profile of KMT2C or KMT2D in primary PCa (up panel) and SU2C-derived metastatic PCa datasets (down panel). The blue arrow indicated the approximate amino acids (AA) locations targeted by CRISPR/Cas9 guide for KMT2C or KMT2D. (C). Indel frequencies for KMT2C and KMT2D in BPH1 cell clones mutated by CRISPR/Cas9.

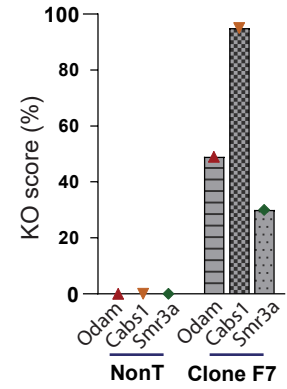
A



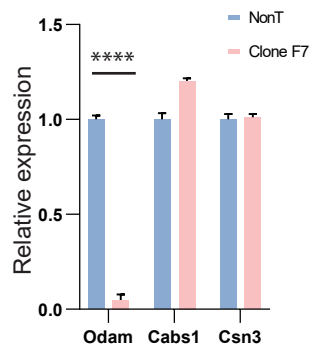
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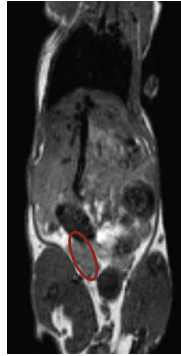
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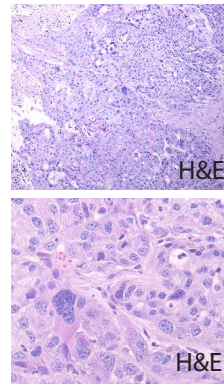
D



E



F



G

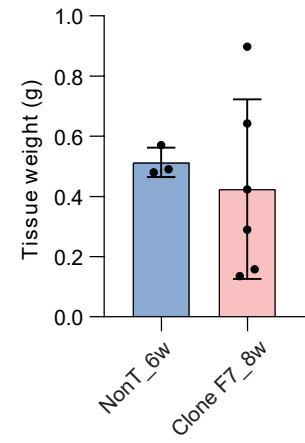


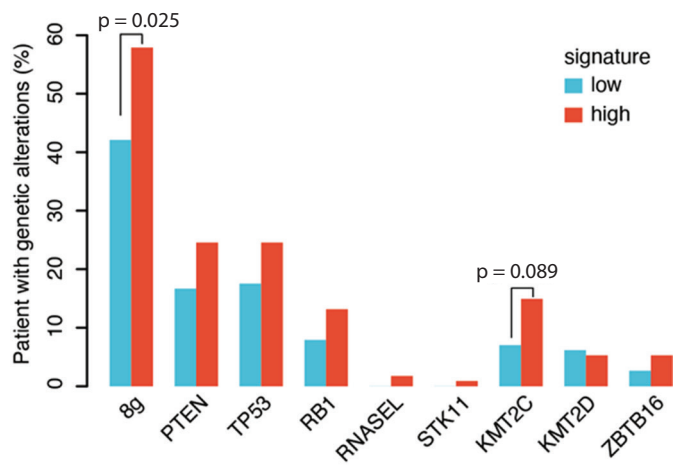
Figure S14: Disruption of Odam and Cabs1 impairs metastatic formation.

A) Representative pictures of a lung metastatic cell line derived from 8g PCa. B) The indel frequency for the eight target genes in the established cell line. C) KO score of the four target genes at the mouse chr5qE1 for clone F7. D) mRNA expression of three target genes in the control cells and clone F7 (n=3, **** = $p < 0,0001$). E) MRi scanning of mice 6 weeks after orthotopically implantation of F7 cells into the prostate. The red dotted line marks tumor formation in the prostatic lobe. A representative picture is shown (n=4). F) H&E staining of the primary PCa 6 weeks after implantation. Representative pictures are shown (n=3). G) Weight of the primary tumor for control cells 6 weeks after implantation and for clone F7 at 8 weeks after implantation (n=3-6).

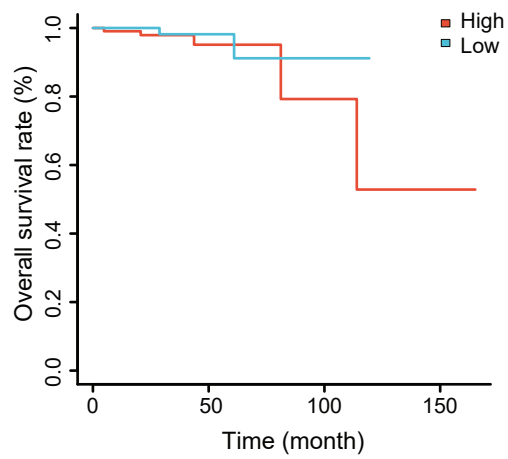
Figure S15: A gene signature was found based on 8g primary tumor alone.

A) The down-regulated genes (n=240), commonly shared by 8g primary and secondary tumor groups, can distinguish human patients with metastases from patients with primary tumors ([GSE35988, n=54, 35](#)). B) The down-regulated genes (n=394) of 8g-derived primary tumor but not present in 5g tumors differentiate human PCa into primary and secondary tumors ([GSE35988, n=54, 35](#)).

A



B



C

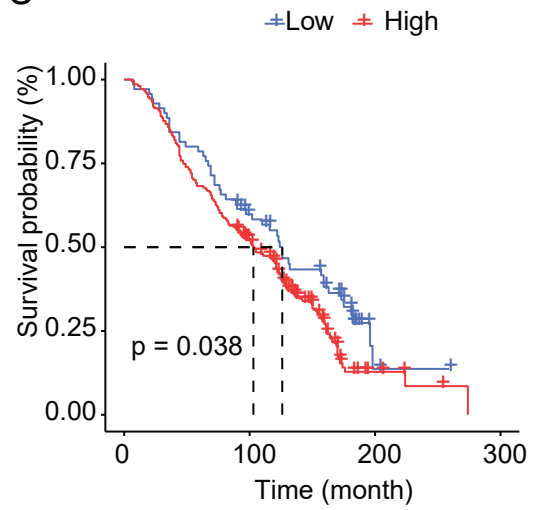


Figure S16: DEGs between 5g and 8g predict overall survival

(A) Expression of 20 signature genes between 5g and 8g was correlated with mutation statuses for the 8 target genes in the TCGA dataset. Samples were grouped into high/low scores in relation to single mutations or mutations in all 8 genes ($p < 0.025$, hypergeometric test). (B) The 20-gene set from the DEGs between 5g and 8g was used to evaluate overall survival for the TCGA dataset ($n=494$). (C) A 10-gene panel derived from the DEGs between 5g and 8g tumors was used to evaluate the survival status in the Swedish Watchful Waiting cohort (GSE16560, $n=280$).