Supplementary Figures

Molecular profiling stratifies diverse phenotypes of treatment-refractory metastatic castration-resistant prostate cancer

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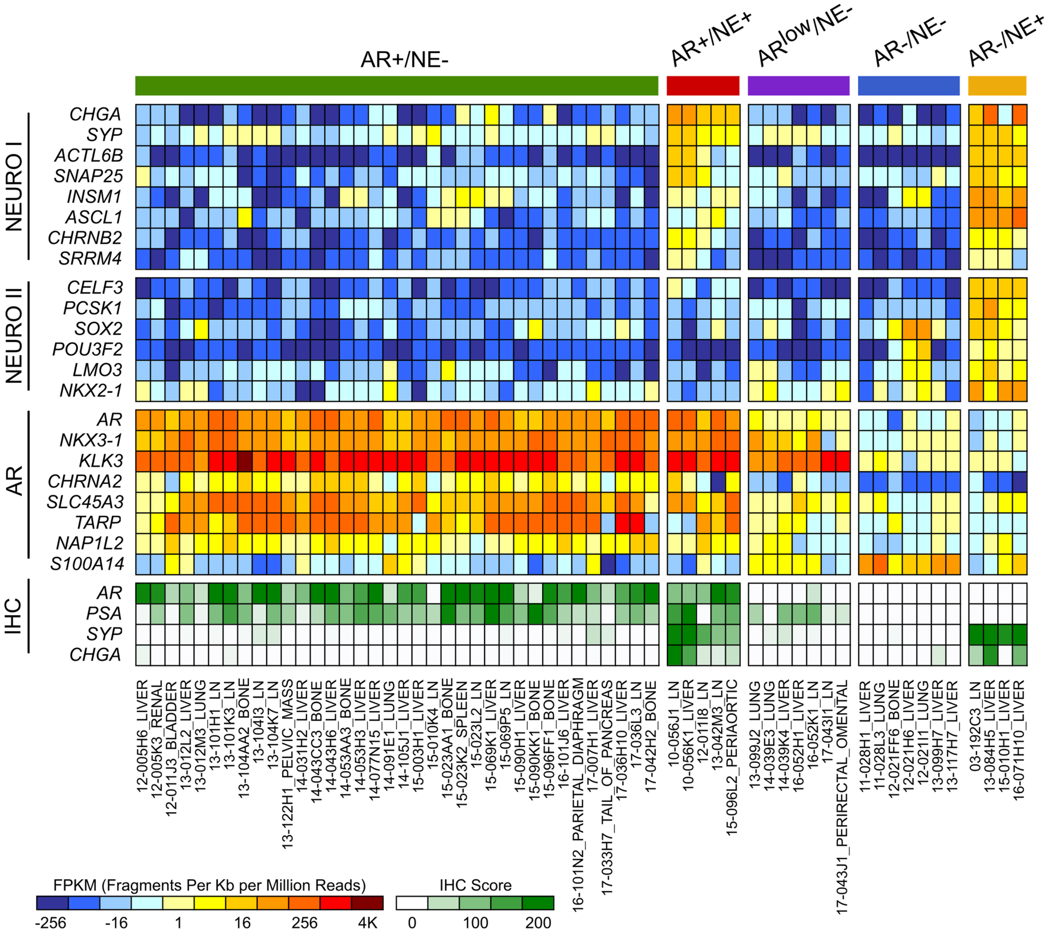
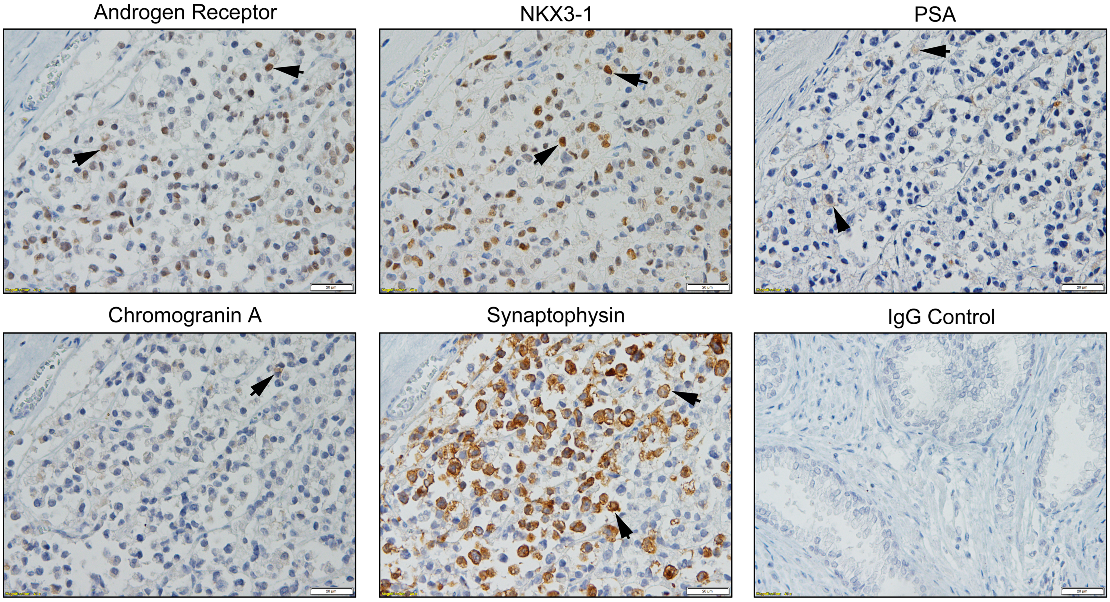


Figure S1. mCRPC tumor phenotype classifications using RNA-Seq and IHC approaches. RNA-Seq heatmap and corresponding IHC data of mCRPC specimens acquired through the rapid autopsy program from 2003-2017 (n=62; modified from Figure 1B). REST-repressed NE genes are listed in the NEURO I panel (top), NE-associated transcription factors are listed in the NEURO II panel (second from top) and AR and AR regulated genes are listed in the AR panel (second from bottom). The IHC analysis (bottom) was conducted on adjacent tumor tissue from the specimen used for RNA-Seq. Results are expressed as $\log_2$ Fragments Per Kilobase of transcript per Million mapped reads (FPKM) or as IHC scores from 0-200 and colored according to scale.



Androgen Receptor

NKX3-1

PSA

Chromogranin A

Synaptophysin

IgG Control

Figure S2. Intratumoral heterogeneity. Androgen receptor, NKX 3-1, PSA, chromogranin A, and synaptophysin expression were assessed in a single core from a lymph node metastasis from a prostate cancer rapid autopsy patient. IgG control was assessed in prostate. Arrows indicate positive staining cells. Bar = 20 microns.

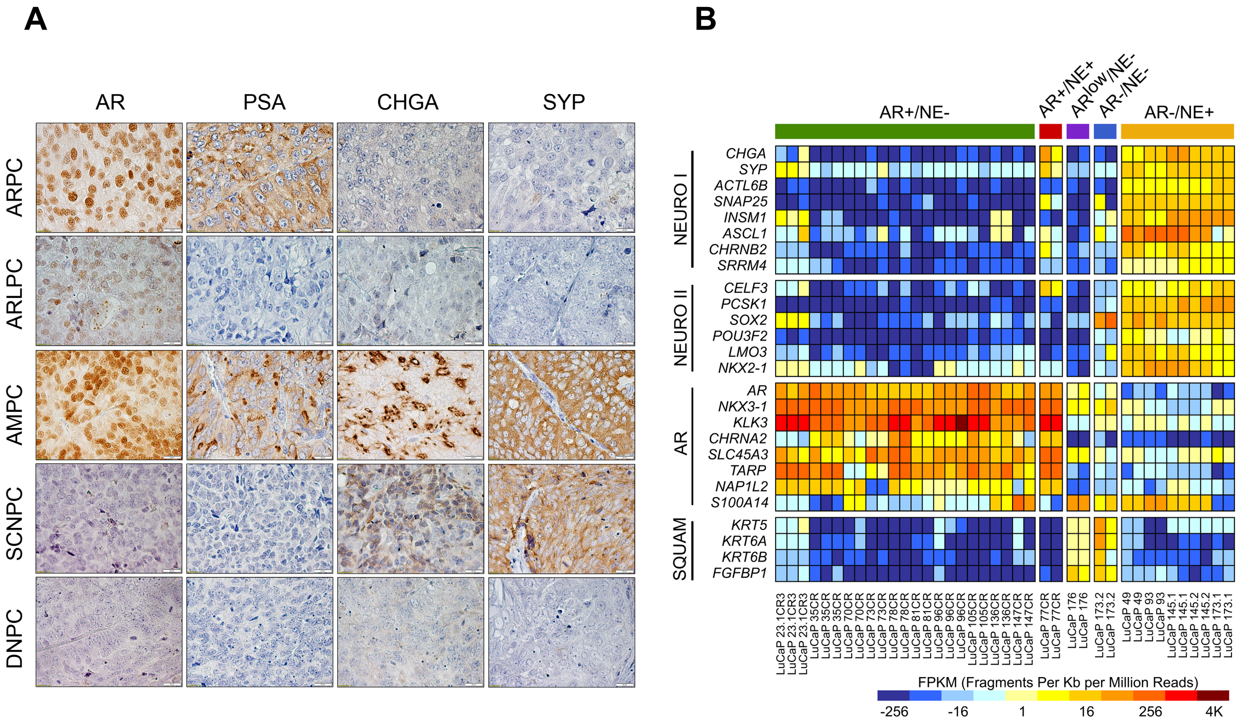


Figure S3. The molecular heterogeneity of mCRPC is reflected in LuCaP PDX models. (**A**) Immunohistochemistry of LuCaP PDX specimens representing five different phenotypes. Primary antibodies were directed to AR, PSA, CHGA and SYP. (**B**) RNA-Seq heatmap of the LuCaP CR PDX series (ARPC), LuCaP 77CR (AMPC), LuCaP 176 (ARLPC), LuCaP 173.2 (DNPC), and the LuCaP SCNPC series. NE genes listed in the NEURO I panel (top) are expressed in the AMPC phenotype whereas NE genes from both NEURO I and NEURO II (second from top) are expressed in SCNPC. Genes regulated by AR are listed in the AR panel (second from bottom) and genes associated with squamous cell carcinoma are listed in SQUAM (bottom panel). Biological replicates from 2-3 tumors per model are shown. Results are expressed as $\log_2$ FPKM and colored according scale. ARPC (AR-high PC; AR+/NE-), ARLPC (AR-low PC; AR^{low}/NE-), AMPC (amphicrine PC; AR+/NE+), DNPC (double-negative PC; AR-/NE-) and SCNPC (small cell or neuroendocrine PC; AR-/NE+).

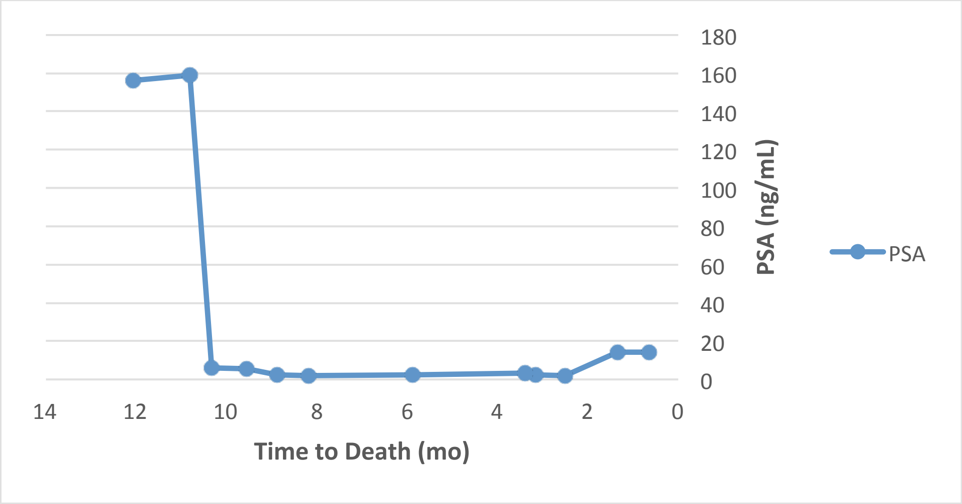


Figure S4. PSA test results from patient 13-084 while on treatment. Serum PSA levels from patient 13-084 were determined through a Hybritech Assay at the University of Washington Medical Center.

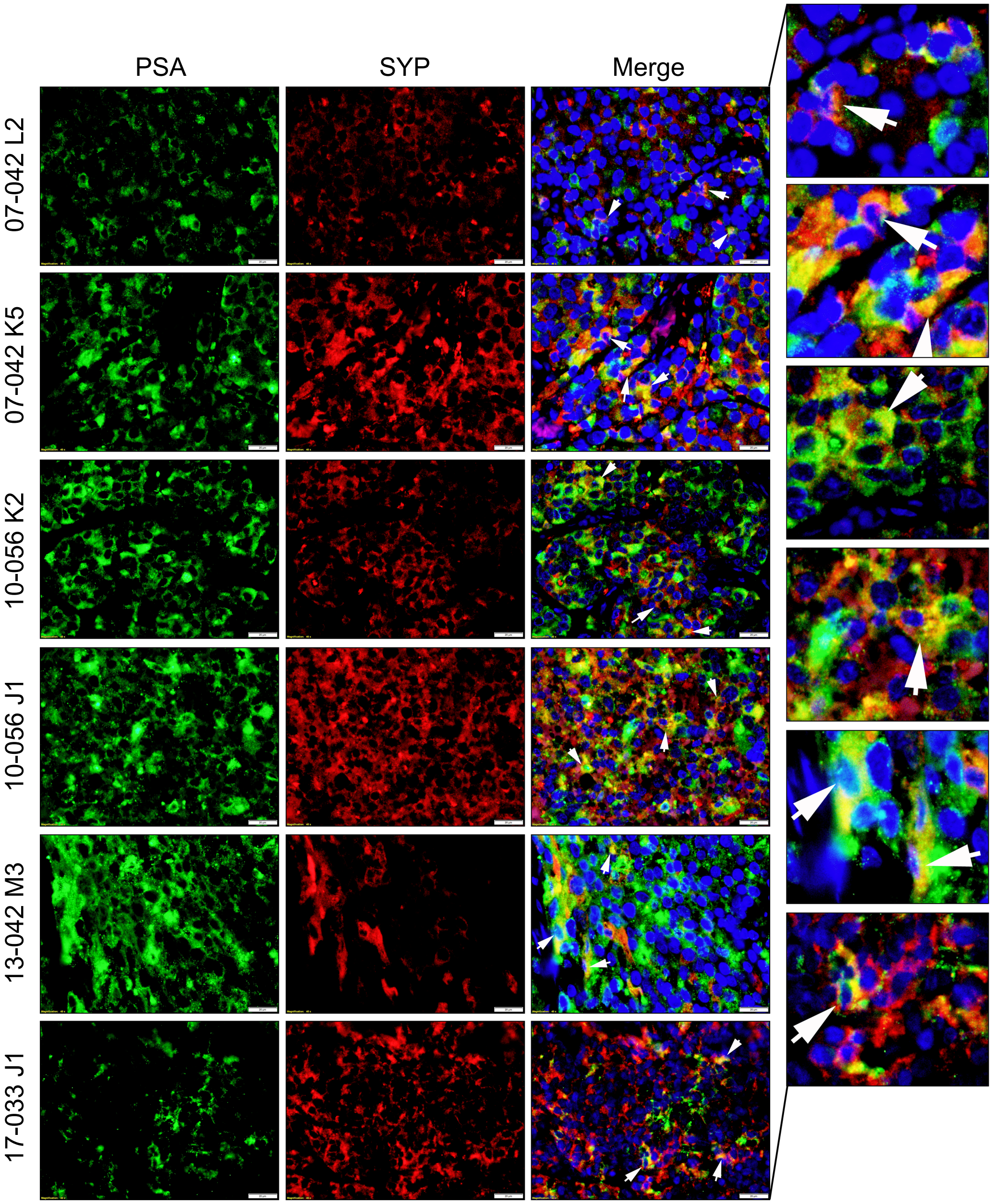


Figure S5. PSA and SYP co-expression in patient samples. Immunofluorescence of amphicrine/mixed patient tumor sections using primary antibodies directed to PSA (green) and SYP (red). Arrows (white) in the merge panels indicate tumor cells that co-express PSA and SYP (yellow to orange). Sections were counterstained with DAPI (blue) and correspond to the same tumor regions used for RNA Sequencing. Bar = 20 microns.

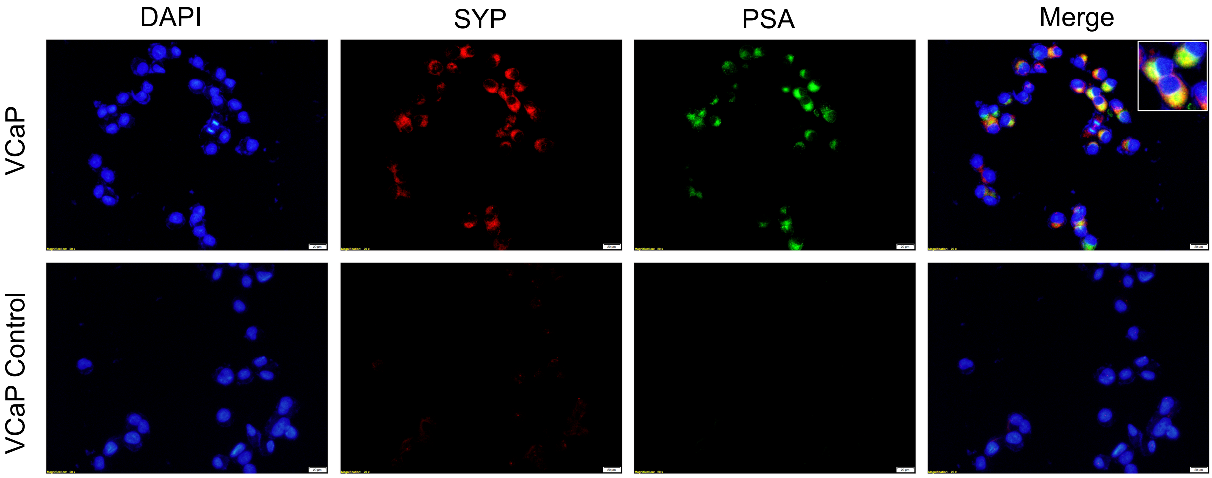


Figure S6. VCaP cells are models of amphicrine PC. Immunofluorescence of VCaP cells using primary antibodies directed to PSA (green) and SYP (red). Cells that co-express PSA and SYP (yellow to orange) are shown in the merge panels. Cells were counterstained with DAPI (blue) and control slides were stained with secondary antibody only (bottom panels). Bar = 20 microns.

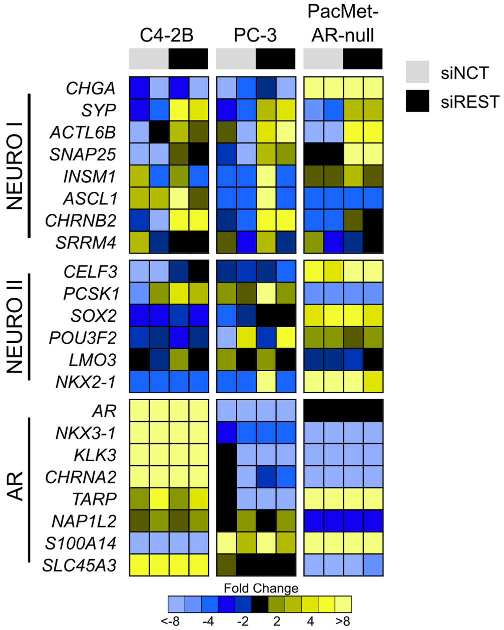


Figure S7. REST knockdown upregulates NEURO I genes in cell lines. RNA-Seq heatmap of C4-2B, PC-3 and PacMet AR-null cells transfected with siNCT or siREST illustrating NE associated genes (NEURO I and NEURO II) and AR and AR regulated genes. Log2 mean-centered ratios of genes are colored according to scale.

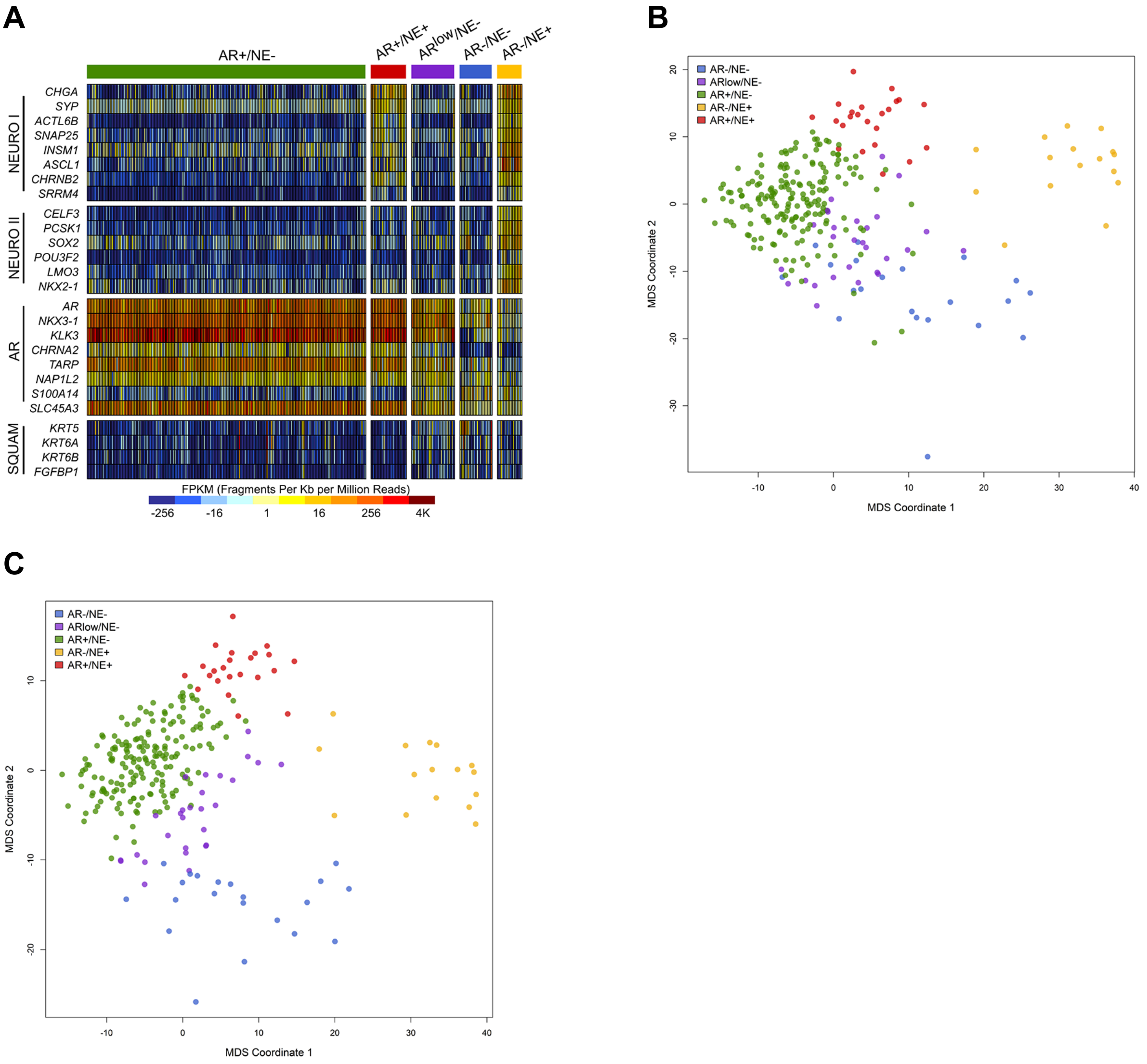


Figure S8. The 26-gene transcriptional signature discriminates the five mCRPC phenotypes in the SU2C cohort. (A) RNA sequencing heatmap and (**B**) MDS and cluster analysis of mCRPC specimens from the SU2C cohort (n=270) using the proposed 26-gene transcriptional signature. **(C)** MDS and cluster analysis of mCRPC specimens from the SU2C cohort (n=270) using a 22-gene transcriptional signature that excludes the SQUAM associated genes (*KRT5, KRT6A, KRT6B* and *FGFBP1*). The analysis was conducted on the PolyA RNA-Seq landscapes. ARPC (AR+/NE-; green), ARLPC (AR^{low}/NE-; purple), DNPC (AR-/NE-; blue), AMPC/mixed (AR+/NE+; red), SCNPC (AR-/NE+; yellow).

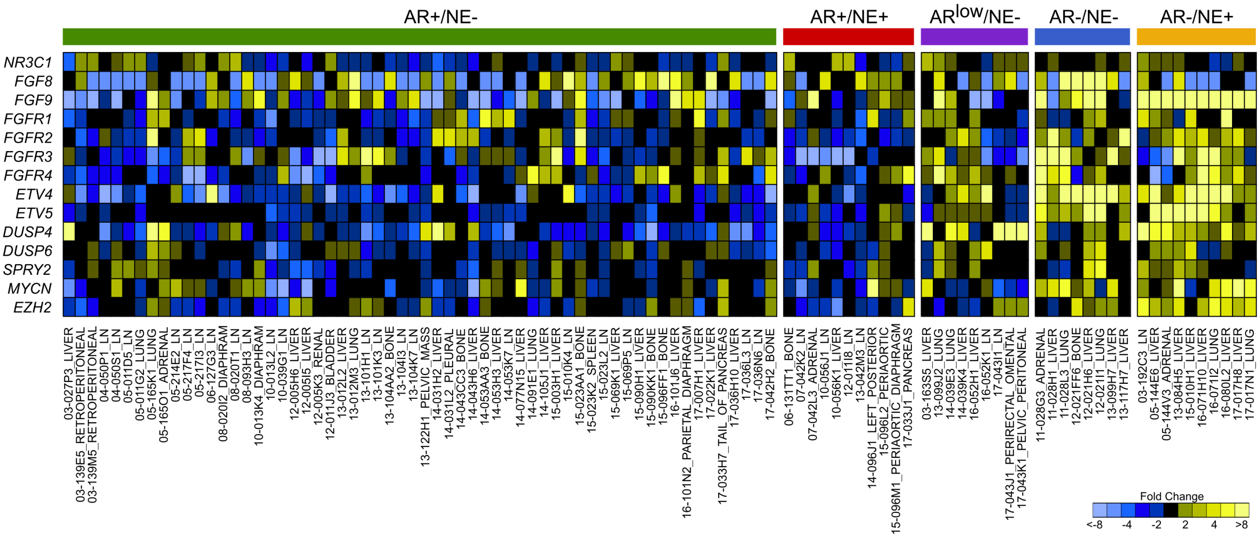


Figure S9. Gene expression profiles of factors that identify mCRPC phenotypes. RNA-Seq heatmap of mCRPC specimens acquired through the rapid autopsy program from 2003-2017 (n=98). Results are expressed as mean centered values and colored according scale. ARPC (AR-high PC; AR+/NE-), ARLPC (AR-low PC; AR^{low}/NE-), AMPC (amphicrine PC; AR+/NE+), DNPC (double-negative PC; AR-/NE-) and SCNPC (small cell or neuroendocrine PC; AR-/NE+).

Supplementary Table S1. Antibodies used for immunohistochemistry (IHC) and immunoblot (IB)

Protein	Company	Product #	Dilution
anti-Androgen Receptor	Biogenex	MU256-UC	IHC (1:60), IB (1/1000)
anti-Prostate-specific Antigen	Dako	A0562	1:1000
anti-Chromogranin A	Dako	M0869	1:100
anti-Synaptophysin	Santa Cruz	sc-17750	IHC (1:200), IB (1/2000)
anti-Cytokeratin 6	Abcam	ab18586	1:10
anti-REST	LS Bio	LS-B15559	1:5000
anti-β-actin	SIGMA	A2228	1:5000
goat anti-rabbit IgG-HRP	Cell Signaling	7074	1:5000
goat anti-mouse IgG-HRP	Cell Signaling	7076	1:5000
goat anti-rabbit IgG- AlexaFluor 488	Invitrogen	A11008	1:400
goat anti-mouse IgG- AlexaFluor 568	Abcam	ab175701	1:400

Supplementary Table S2. Patient demographics

Patient Characteristics	
No. pts.	55
Median age at diagnosis in years (SD)	64 (8.3)
Median age at death in years (SD)	70 (9.2)
Median survival after diagnosis in years (range)	8 (1-25)
Median PSA at death in ng/mL (range)	288.1 (0.2-5690.8)
Gleason (range)	6-10
Bone Metastases/Therapies	
No. pts. with clinically detected bone metastases	53
Median survival after first bone metastases in years (range)	1.8 (0.2-9.2)
No pts. receiving bisphosphonate (%)	32 (58%)
Median treatment duration in years (range)	1.2 (0.1-9.1)
Androgen Ablation Therapy	
No. pts. receiving androgen ablation (%)	54 (98%)
Median treatment duration in years (range)	4.2 (0.3-15.1)
No. pts receiving ABI, ENZ or Both	8, 4, 17
Other Therapies	
Ketoconazole (%)	17 (31%)
DES (%)	13 (24%)
Corticosteroids (%)	42 (76%)
Estramustine (%)	3 (5%)
Taxotere (%)	44 (80%)

Selected clinical data and treatment information for rapid autopsy patients in the study. Pts – Patients.