SUPPPLEMENTARY DATA

Characterization Of HOXB13 Expression Patterns In Localized And Metastatic Castration Resistant Prostate Cancer.

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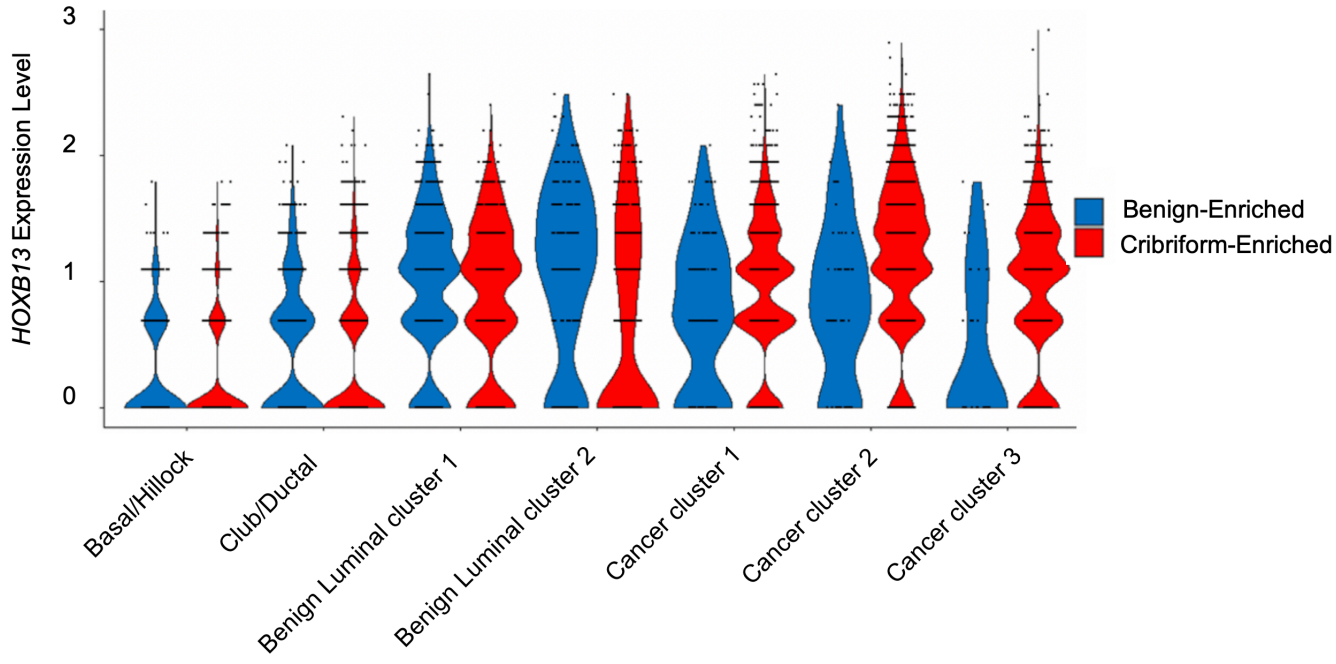
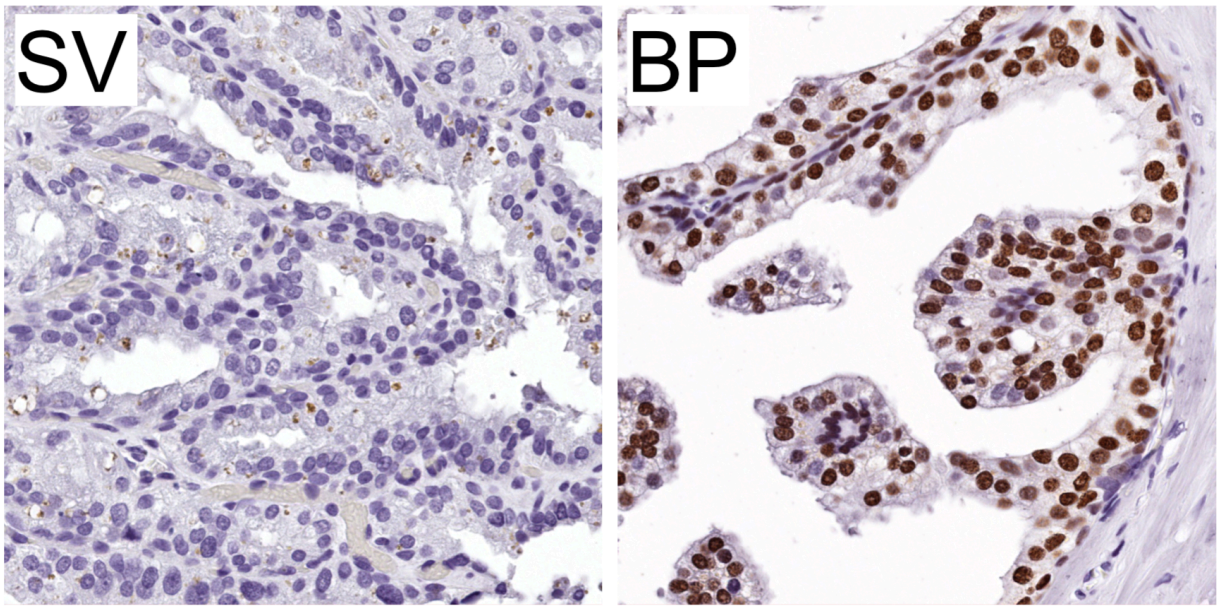
Supplementary Figure 1. *Patel, Sayar et al.*



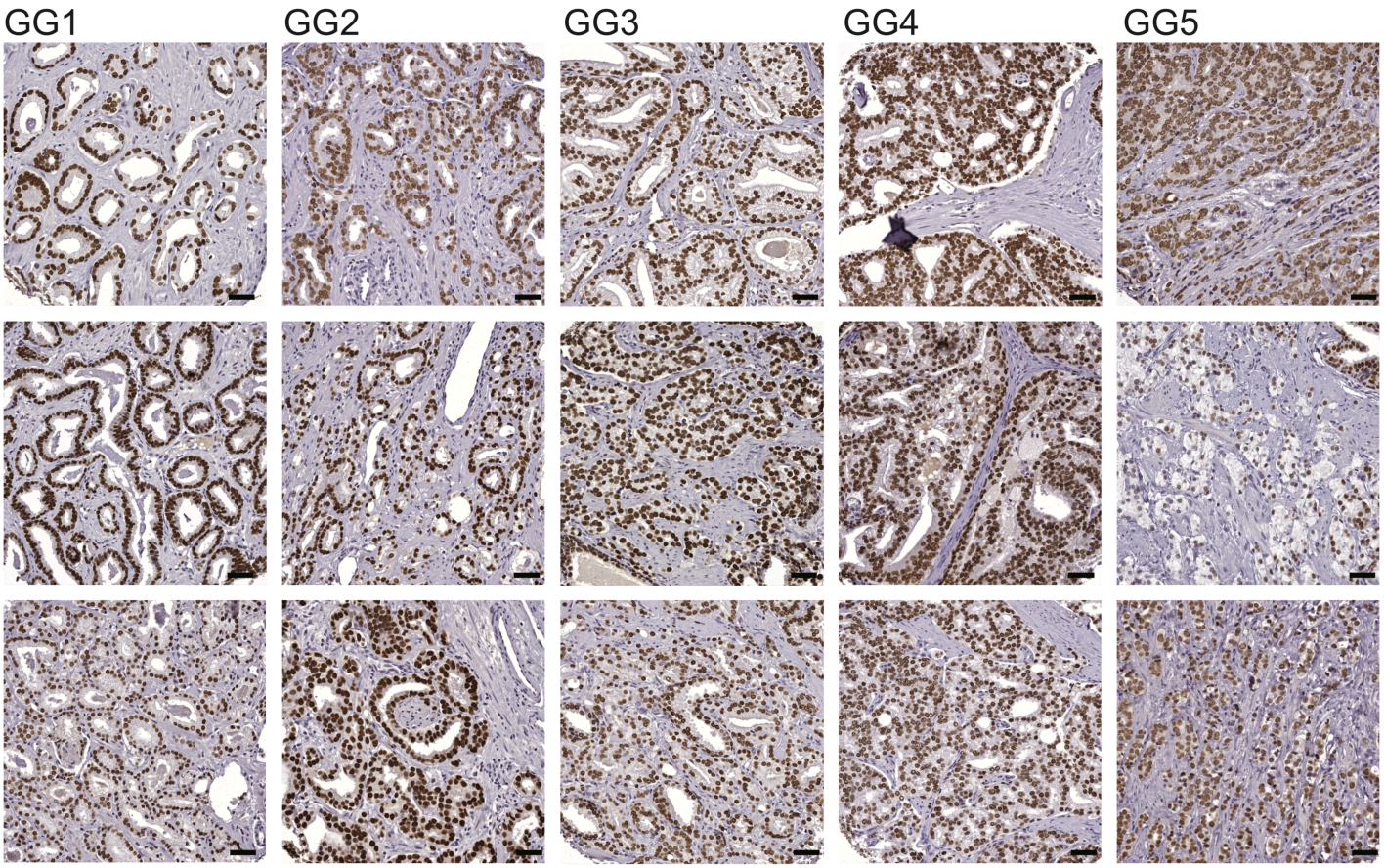
Supplementary Figure 1. Analyses of previously published scRNA-seq data (PMID: 36229464) from radical prostatectomy specimens reveals high levels of HOXB13 RNA expression in both benign and cancer luminal epithelial cells and lower expression in basal/Hillock and club/ductal cells.

Supplementary Figure 2. *Patel, Sayar et al.*



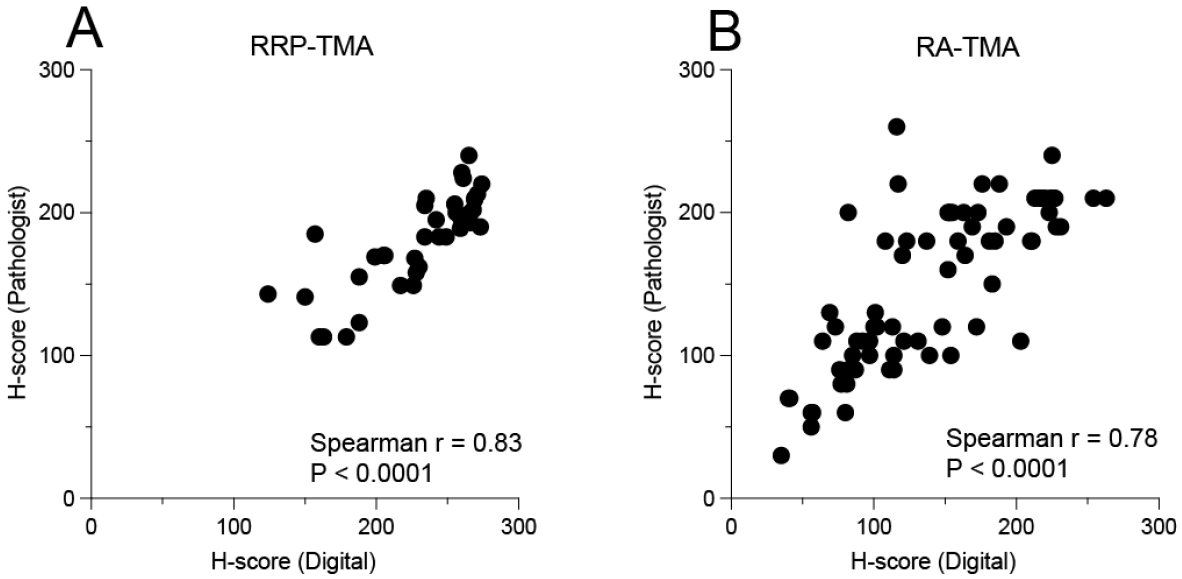
Supplementary Figure 2. HOXB13 is expressed in benign human prostate epithelium (BP), but not in seminal vesicle/ejaculatory duct tissue.

Supplementary Figure 3. *Patel, Sayar et al.*



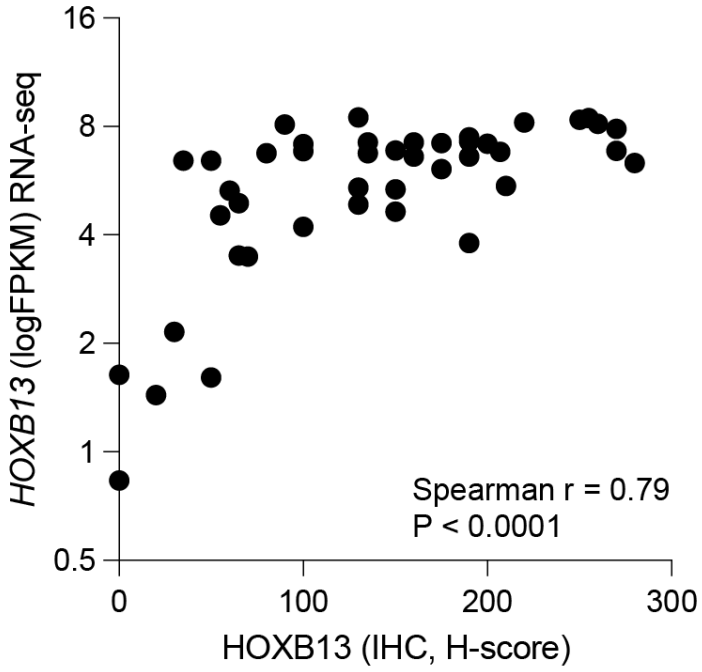
Supplementary Figure 3. Representative micrographs of HOXB13 IHC across different grade groups in the radical prostatectomy cohort. Scale bar indicated 50□m.

Supplementary Figure 4. *Patel, Sayar et al.*



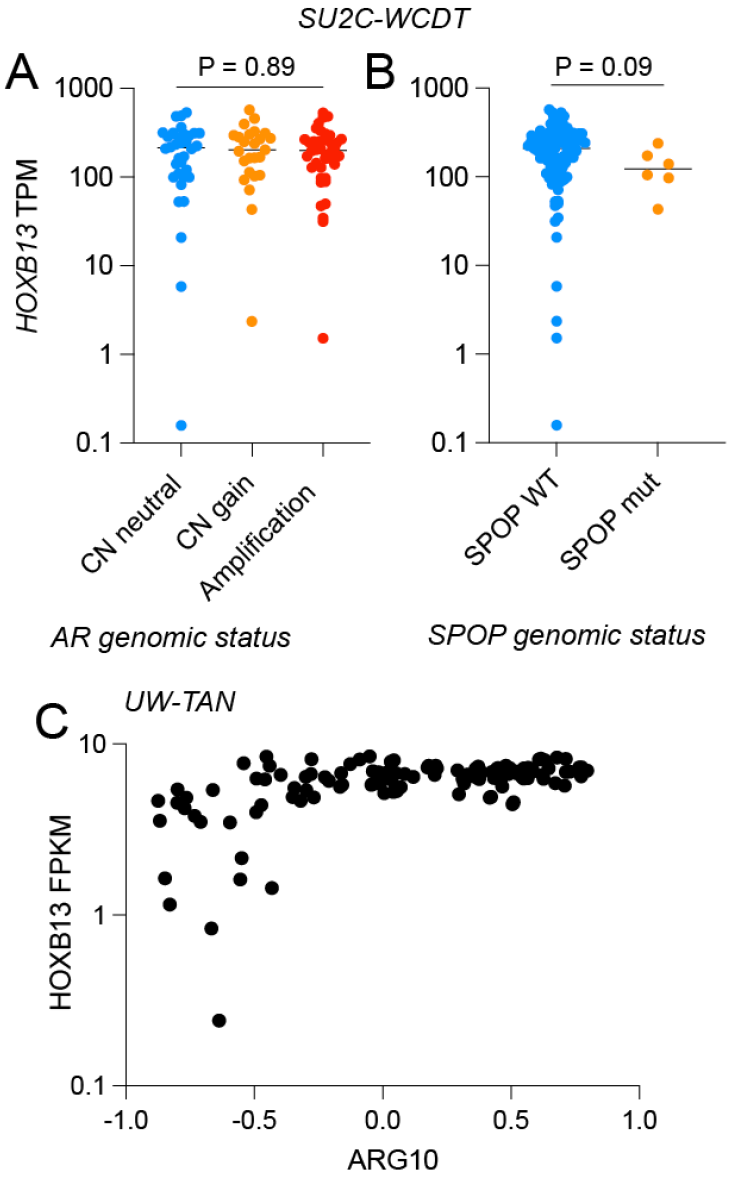
Supplementary Figure 4. Correlation between semiquantitative, expert pathologist determined HOXB13 protein expression H-score (Pathologist, y-axis) and quantitative digital image analysis-based HOXB13 H-scores (Digital, x-axis) for N=38 matched localized (A) and matched N=71 metastatic (B) tumor samples. For digital image analysis, Digitized slides were analyzed with QuPath-0.4.3.

Supplementary Figure 5. *Patel, Sayar et al.*



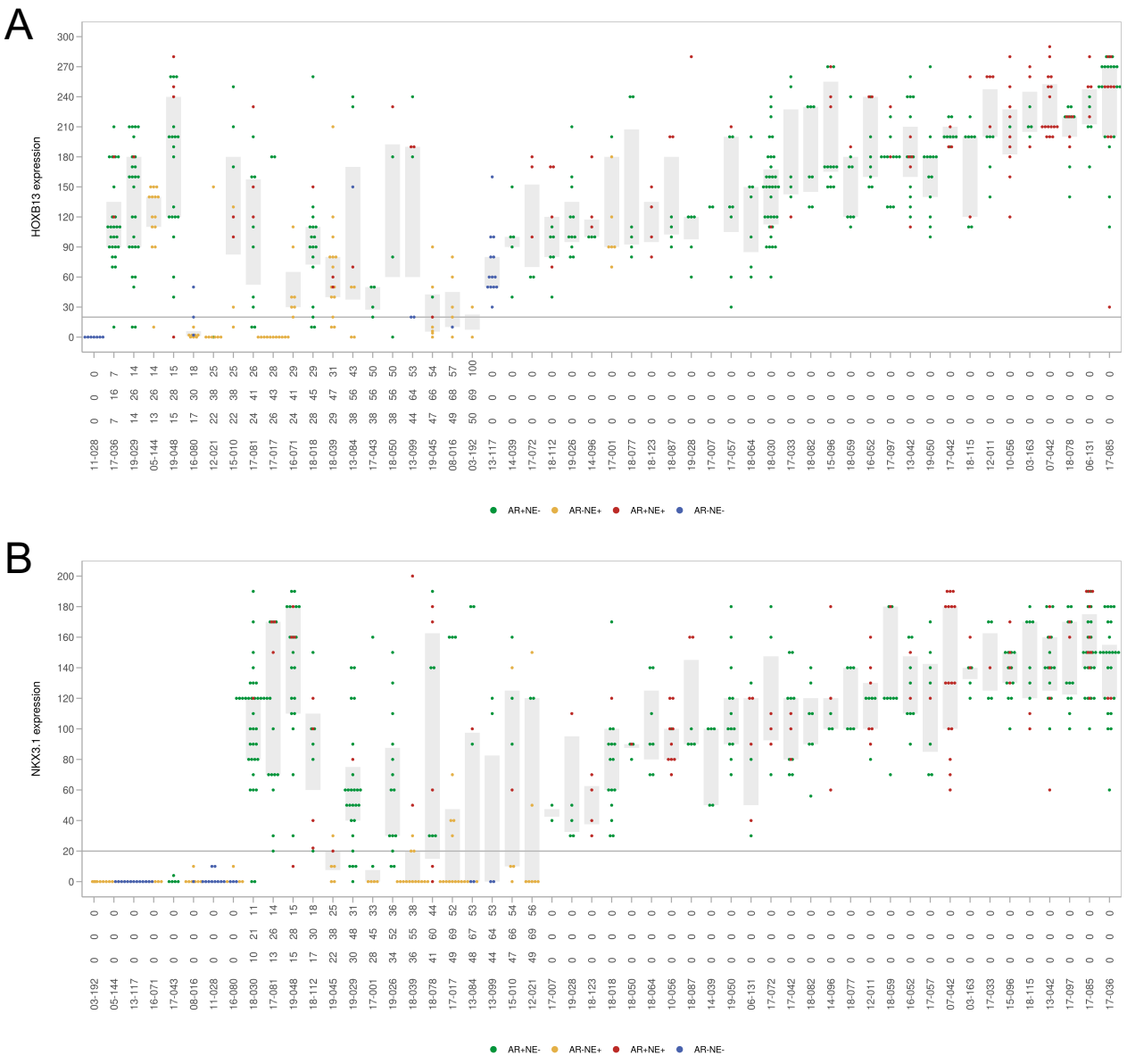
Supplementary Figure 5. Correlation between HOXB13 mRNA levels (determined by RNA-seq) and HOXB13 protein expression levels (determined by semiquantitative IHC) on 52 matched mCRPC metastasis samples.

Supplementary Figure 6. *Patel, Sayar et al.*



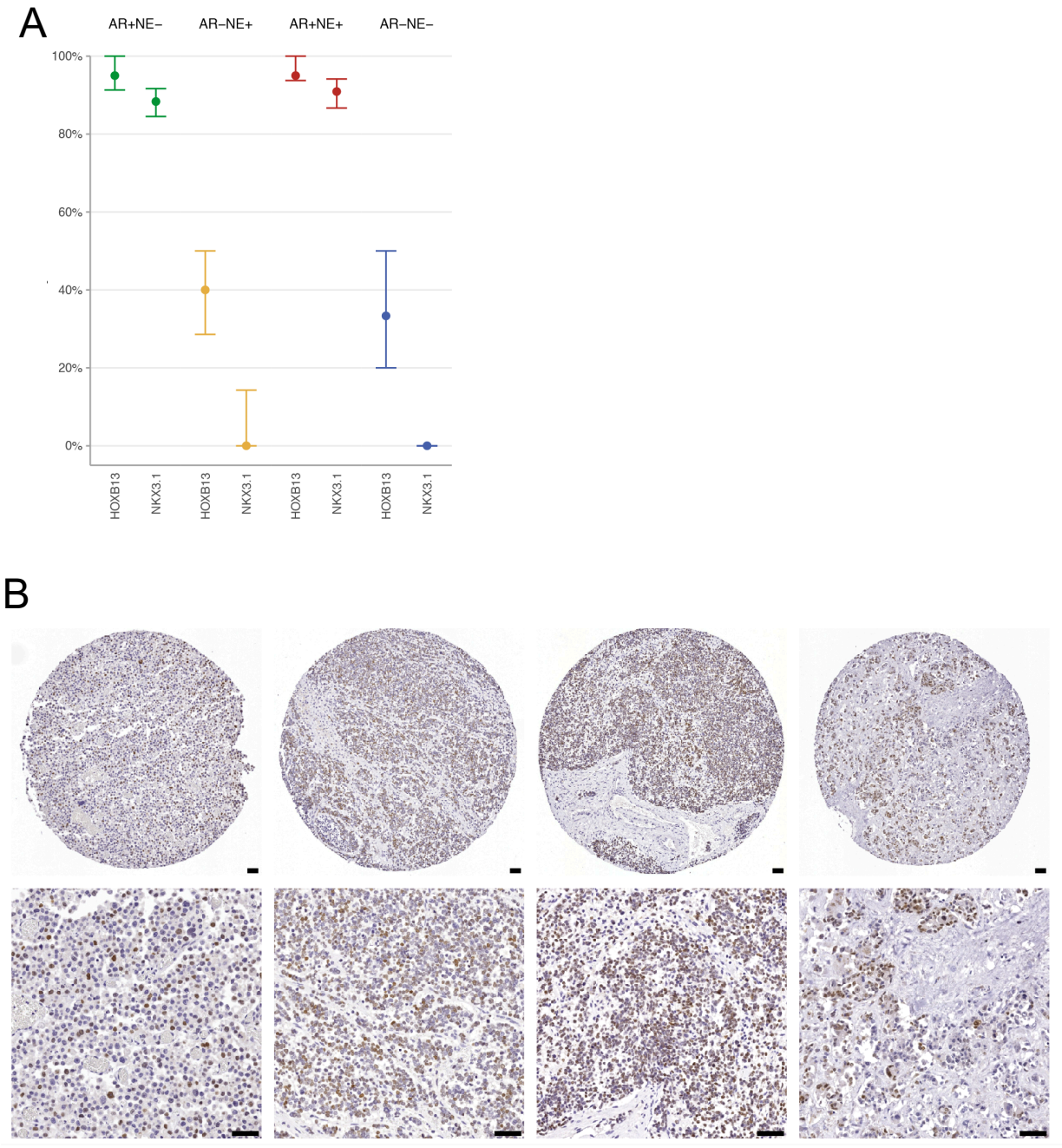
Supplementary Figure 6. Correlation of HOXB13 expression with genomic features. **A.** HOXB13 expression in the SU2C-WCDT (PMID: 30033370) cohort in cases with no AR locus alteration and cases with low level copy-number gain and high-level amplification. **B.** HOXB13 expression in SPOP wild type and SPOP mutant cases. **C.** Association between HOXB13 expression and AR signaling activity (as determined by the ARG10 score) in the UW-TAN cohort.

Supplementary Figure 7. *Patel, Sayar et al.*



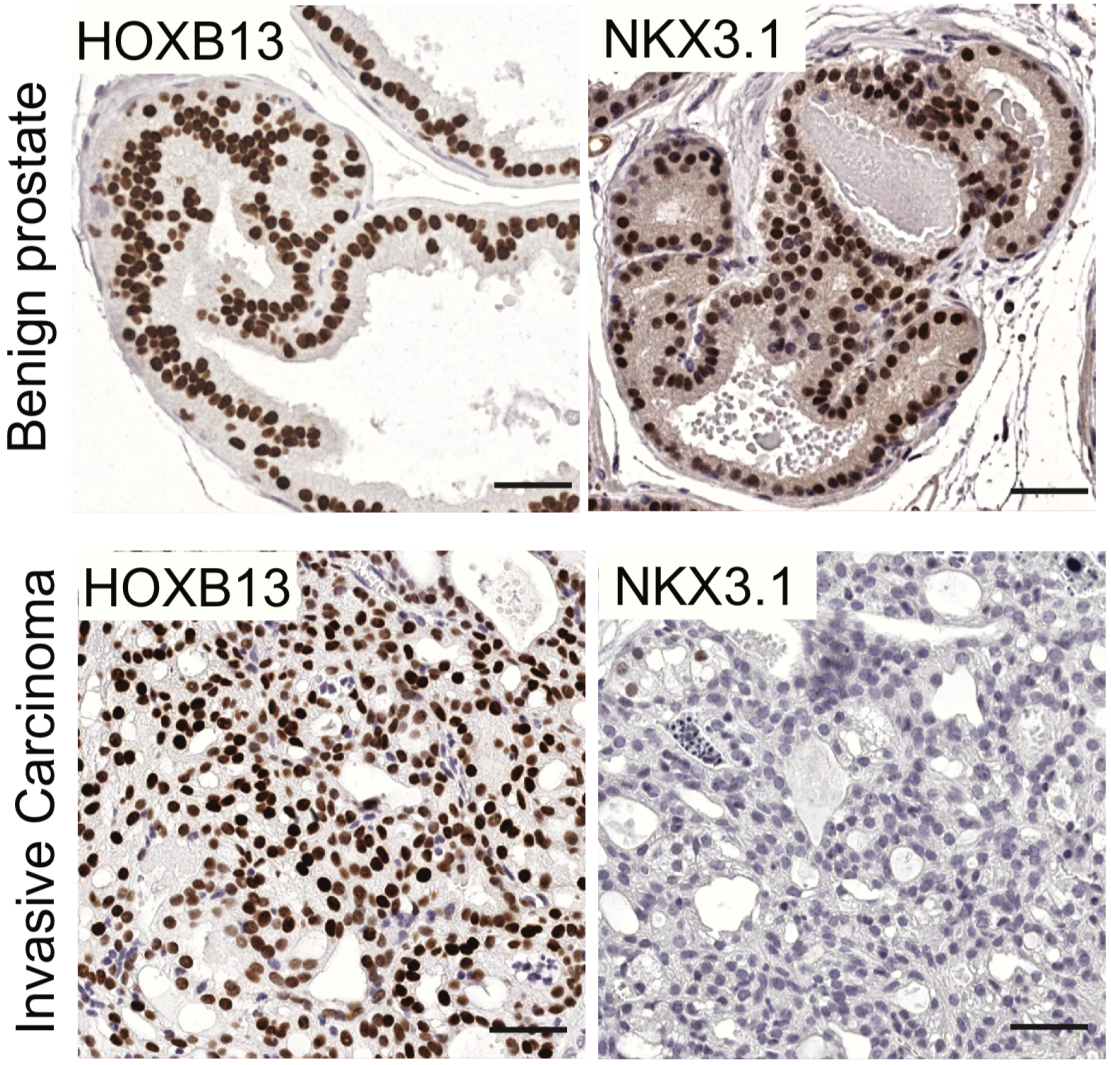
Supplementary Figure 7. **A.** Dot and box plot show the distribution of HOXB13 protein expression H-scores in 52 cases from the UW-TAN rapid autopsy cohort. Each dot represents a tumor sample; the color codes indicate the molecular subtype (AR+/NE- [green], AR-/NE+ [yellow], AR+/NE+ [red] and AR-/NE- [blue]). Gray shadings show inter-quartile ranges. **B.** Dot and box plot show the distribution of NKX3.1 protein expression H-scores. Numbers below graph indicate Shannon, Simpson and hypergeometric diversity indices.

Supplementary Figure 8. *Patel, Sayar et al.*



Supplementary Figure 8. **A.** Probability of positive reactivity (H-score ≥ 40) for HOXB13 and NKX3.1 across different molecular subtypes. **B.** Representative micrographs of tumors with an HOXB13 H-score of 40. Scale bar indicates 50 μm.

Supplementary Figure 9. *Patel, Sayar et al.*



Supplementary Figure 9. IHC for HOXB13 and NKX3.1 in benign murine prostate tissue and SKO ($Pten^{-/-}$, age 61 weeks). Note the loss of NXK3.1 expression in $Pten^{-/-}$ adenocarcinoma compared to the retained high expression of HOXB13. Scale bar indicates 50 μm.

Supplementary Table 1. *Patel, Sayar et al.*

Characteristic	N = 297
Age, median (IQR)	59 (55, 63)
Unknown	16
Race, n (%)	
African-American	22 (7.9%)
Caucasian	239 (85%)
Other	19 (6.8%)
Unknown	17
PSA, median (IQR)	6.7 (5.0, 9.4)
Unknown	19
NCCN risk group, n (%)	
low risk	88 (32%)
intermediate risk	138 (51%)
high risk	45 (17%)
Unknown	26
Gleason Sum, n (%)	
6	1 (0.4%)
3+4	103 (37%)
4+3	102 (36%)
8	52 (19%)
9-10	23 (8.2%)
Unknown	16
Stage, n (%)	
organ confined	127 (45%)
extraprostatic extension	114 (41%)
seminal vesicle involvement	25 (8.9%)
lymph node positive	14 (5.0%)
Unknown	17

Supplementary Table 1. Baseline clinicopathological features of the radical prostatectomy cohort.

Supplementary Table 2. *Patel, Sayar et al.*

Characteristic	N	Beta	95% CI[1]	p-value
AGE	281	-0.11	-0.69, 0.47	0.7
YEAR	281			
1997-2000		—	—	
2001-2005		-2.8	-13, 7.0	0.6
RACE	280			
White		—	—	
Black		-14	-27, -0.81	0.037
Other		2.4	-11, 16	0.7
FAMILY_HISTORY	226			
No		—	—	
Yes		3.6	-4.8, 12	0.4
BMI	271	-0.54	-1.5, 0.44	0.3
PSA	278	-0.51	-1.1, 0.05	0.074
NCCN	271			
low risk		—	—	
intermediate risk		2.7	-5.2, 11	0.5
high risk		-4.6	-15, 6.0	0.4
GLEASON GRADE	281			
9-10		—	—	
6		12	-47, 71	0.7
3+4		24	11, 38	<0.001
4+3		21	8.0, 35	0.002
8		15	0.32, 29	0.045
STAGE	280			
organ confined		—	—	
extraprostatic extension		-1.2	-8.8, 6.4	0.8
seminal vesicle involvement		2.9	-10, 16	0.7
LYMPH NODE POSITIVE		-9.8	-26, 6.8	0.2
ADJUV_TX	212			
none		—	—	
adjuvant radiation only		-28	-62, 6.2	0.11
adjuvant radiation+ADT		-20	-79, 39	0.5

[1]CI = Confidence Interval

Supplementary Table 2. Correlation of clinicopathological features and HOXB13 expression.

Supplementary Table 3. *Patel, Sayar et al.*

BIOCHEMICAL RECURRENCE-FREE SURVIVAL

Characteristic	N	HR[1]	95% CI[1]	p-value
Gleason Grade	218	1.73	1.37, 2.17	<0.001
HOXB13	218	1.00	0.99, 1.01	0.8

[1]HR = Hazard Ratio, CI = Confidence Interval

METASTASIS-FREE SURVIVAL

Characteristic	N	HR[1]	95% CI[1]	p-value
Gleason Grade	216	2.04	1.37, 3.04	<0.001
HOXB13	216	1.00	0.99, 1.01	0.6

[1]HR = Hazard Ratio, CI = Confidence Interval

Supplementary Table 3. Results from univariate analyses for patients with outcomes data.

Supplementary Table 4. *Patel, Sayar et al.*

Total N = 52	
Demographics data	
Age at initial diagnosis (median [IQR])	60.61 [53.92, 66.33]
PSA at diagnosis (median [IQR])	19.50 [7.55, 111.25]
Age at death (median [IQR])	68.63 [61.91, 74.43]
PSA at death (median [IQR])	90.63 [8.69, 1007.51]
Treatment history	
ADT	51 (98)
Abiraterone acetate	33 (65)
Enzalutamide	28 (55)

Supplementary Table 4. Clinical characteristics of patients from the UW-TAN rapid autopsy study with IHC expression data.

Supplementary Table 5. *Patel, Sayar et al.*

Characteristic	N	HR[1]	95% CI[1]	p-value
HOXB13	267	0.98	0.92, 1.04	0.5
Subtype	267			
AR+NE-		—	—	
AR-NE+		5.83	2.88, 11.8	<0.001
AR+NE+		1.40	0.80, 2.45	0.2
AR-NE-		1.24	0.82, 1.88	0.3

[1]HR = Hazard Ratio, CI = Confidence Interval

Supplementary Table 5. Hazard ratios of overall survival after biopsy in the SUC2 cohort.

Supplementary Table 6. *Patel, Sayar et al.*

	HOXB13	PSA	NKX3.1	HMWCK
Prostatic adenocarcinoma	34/34 (100%)	*37/38 (97.4%)*	*35/38 (92.1%)*	*0/38 (0%)*
Urothelial Carcinoma	0/35 (0%)	*0/35 (0%)*	*0/35 (0%)*	*24/35 (68.6%)*
	This study	Ai-Ying Chuang et al.		

Supplementary Table 6. Comparison of HOXB13 and other markers in the differential diagnosis of prostatic adenocarcinoma versus high grade urothelial carcinoma. HOXB13 was assessed as part of this study. PSA, NKX3.1 and HMWCK expression data were acquired on the same case material and were reported previously by Ai-Ying Chuang et al. (PMID: 17667550). Note that 4 prostate cancer cases were not evaluable for HOXB13 due to the absence of tumor cell on deeper TMA sections used in this study. For percentage evaluation, the entire case was considered negative if any TMA spot in a case was not reactive for the respective marker.