

Supplementary Figures

The Homeobox gene, *HOXB13*, Regulates a Mitotic Protein-Kinase Interaction Network in Metastatic Prostate Cancers

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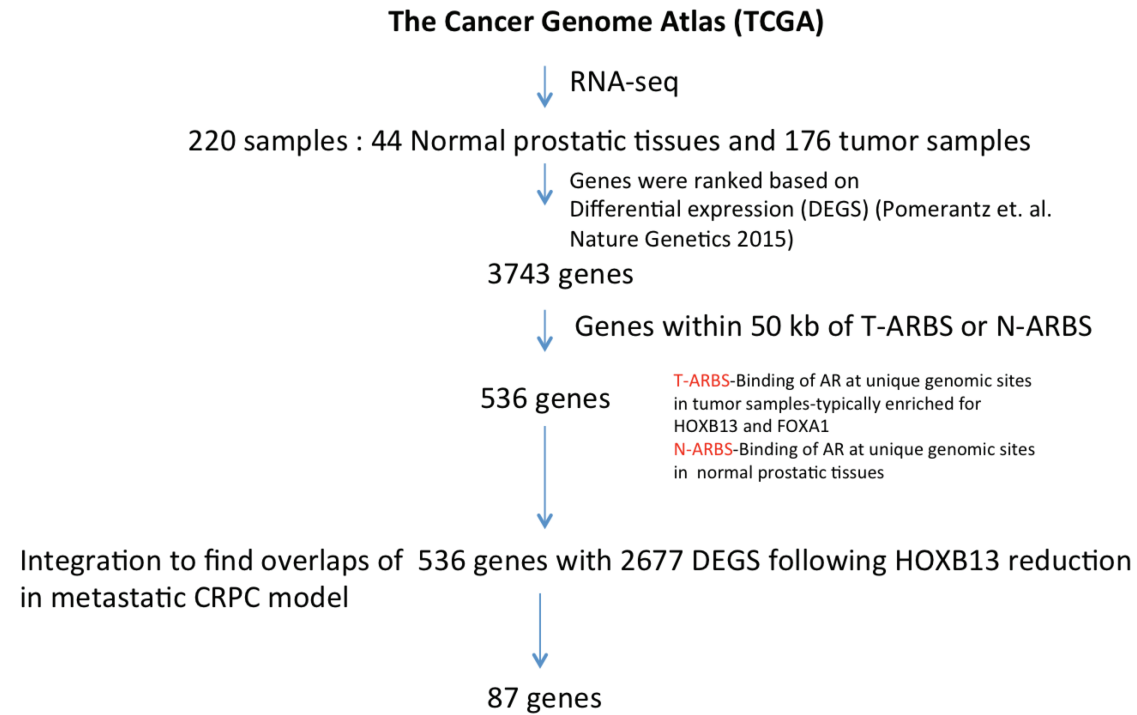
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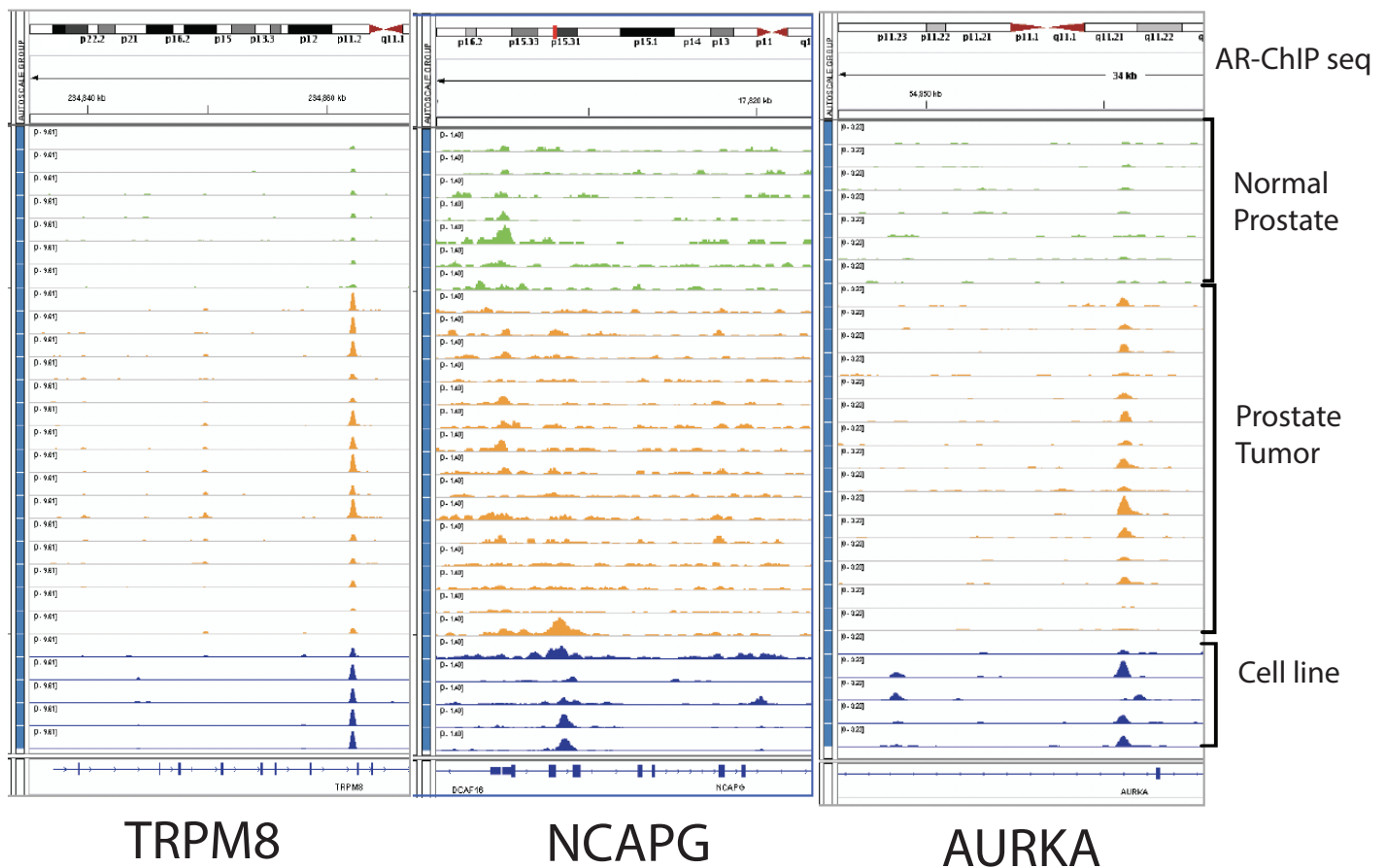
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a. **Flow chart: Identification of the HOXB13 target gene in human prostate tumors**



b

Query DataSets for GSE56288

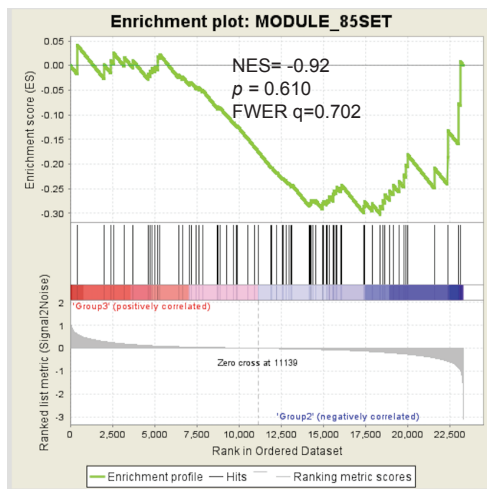


Supplementary Figure 1.

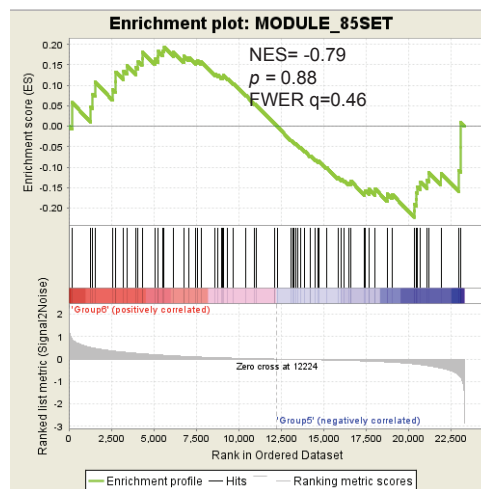
a. Flow chart: Identification of the HOXB13 direct targets in human prostate tumors. Integration of data sets to find overlaps of 536 genes with ~2677 DEGS following HOXB13 reduction in metastatic CRPC model.

b. IGV analysis for AR and HOXB13 binding sites. HOXB13 or AR-ChIP sequencing in GSE56288 for binding in the proximity of the putative AR/HOXB13 target genes in normal prostate (1-7 lines, green peaks), human prostate tumors (8-20 lines, orange peaks) and FOXA1 ChIP-seq in tumor (orange peaks- line 21), HOXB13 ChIP-seq tumor (orange peaks- line 22), HOXB13 ChIP-seq in LNCaP (blue peaks- line 23), AR ChIP-seq in normal prostate cell line LHSAR (expressing HOXB13, FOXA1, HOXB13+FOXA1 (#1) HOXB13 +FOXA1 (#2)) (blue peaks lines, 24-27).

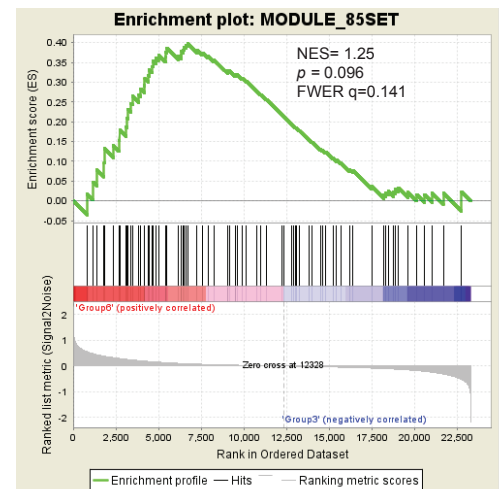
a. NP CRPC vs NP



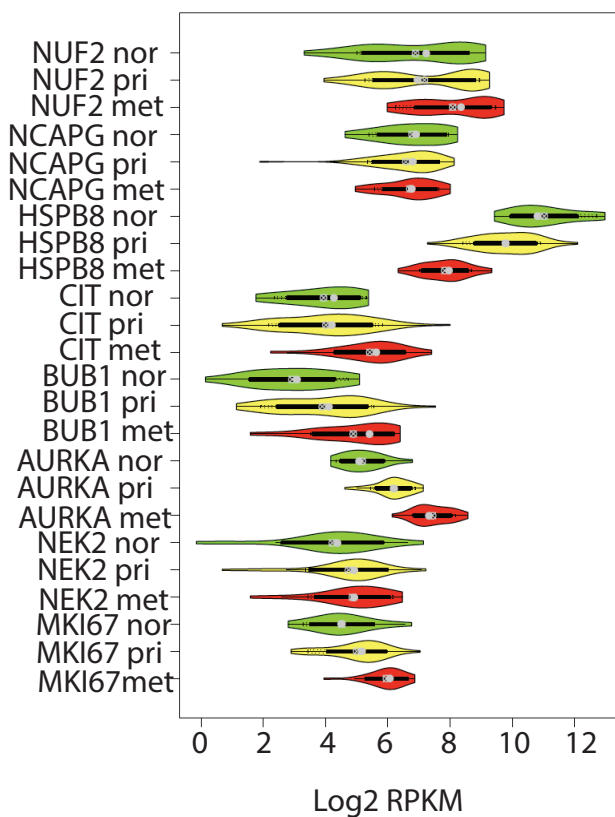
b. NPp53CRPC vs NP 53



c. NPp53CRPC vs NP CRPC

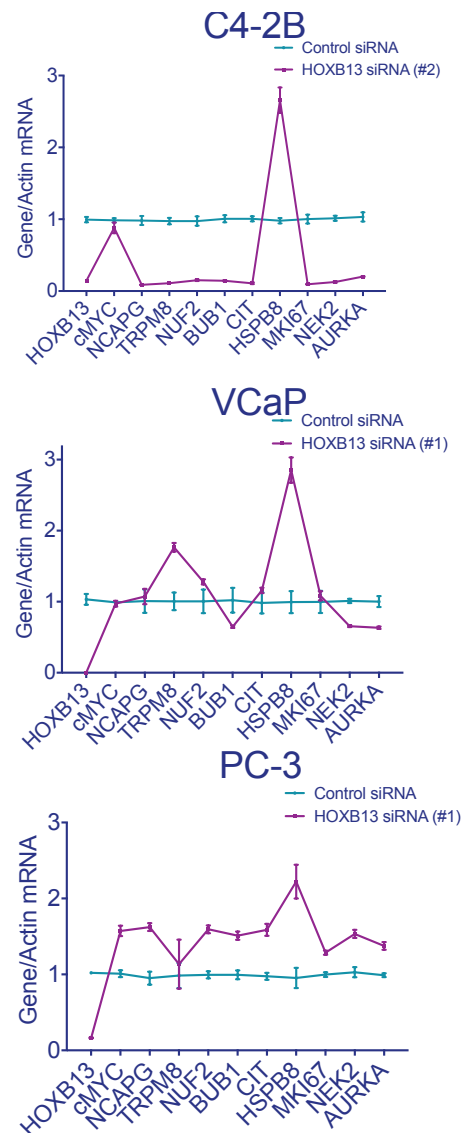


d. GSE 6919



Normal: 233
 Primary PCs: 196
 Metastatic PCs: 75

e.

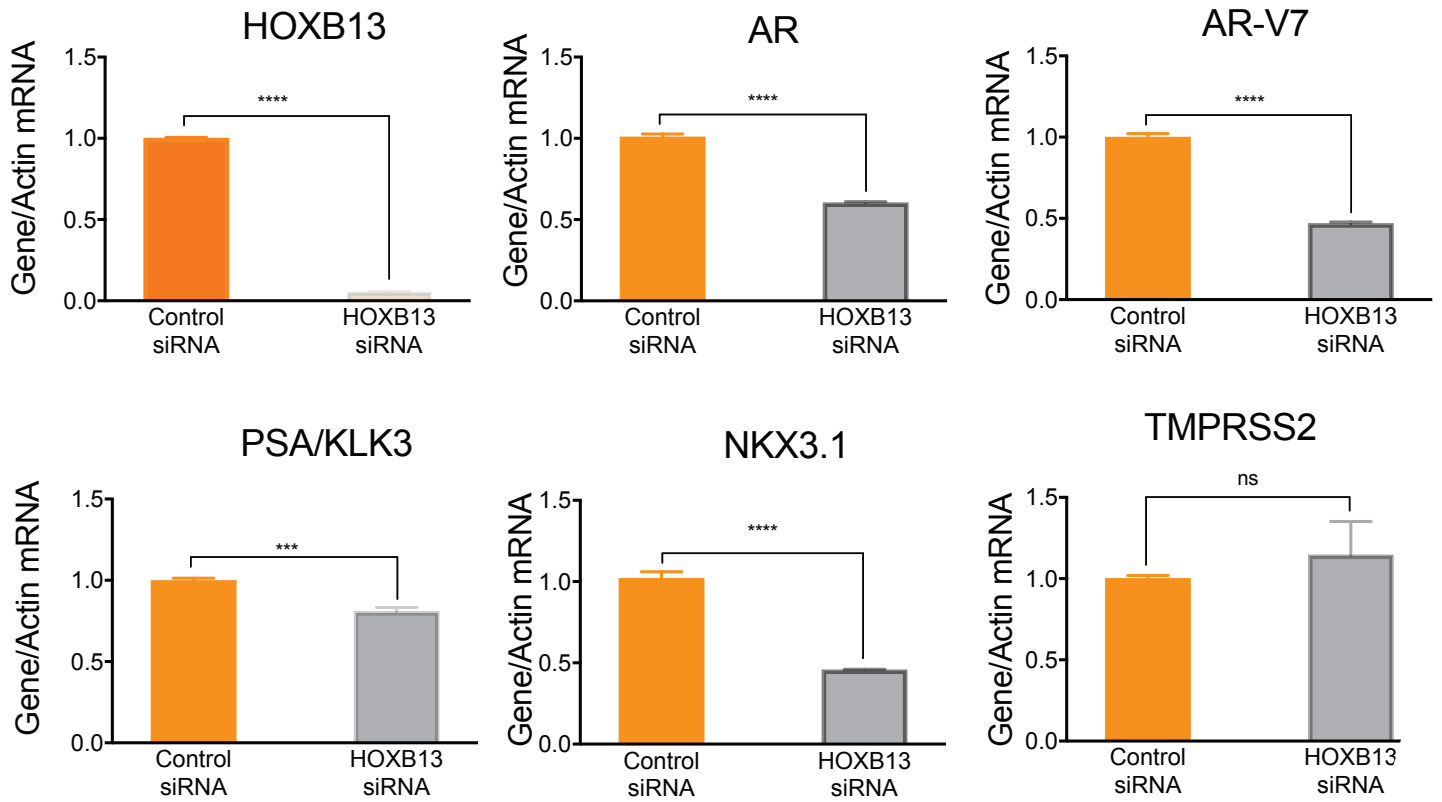


Supplementary Figure 2. HOXB13 transcriptional targets are enriched in high-risk prostate cancers

(a-c) Gene set enrichment analysis comparing HOXB13 target gene expression in intact and castrated (CRPC) *NKX3.I^{haplo}* (N), *NKX3.I^{haplo} PTEN^{cnull}* (NP) and *NKX3.I^{haplo} PTEN^{cnull} p53^{cnull}* (NPp53) GEMMs of prostate adenocarcinoma. **(d)** Violin plots displaying expression of a subset of HOTPAM9 genes in GSE6919 dataset (Chandran U *et al.*, 2007) comprising normal=76; primary=66 and mPCs=25. *p* values (shown in supplementary table). **(e)** C4-2B, VCaP and PC3 cells were transfected with control or HOXB13 siRNA. Total RNA was isolated, followed by qRT-PCR with HOXB13, c-MYC and HOTPAM9 primers. Actin is used as a normalization control.

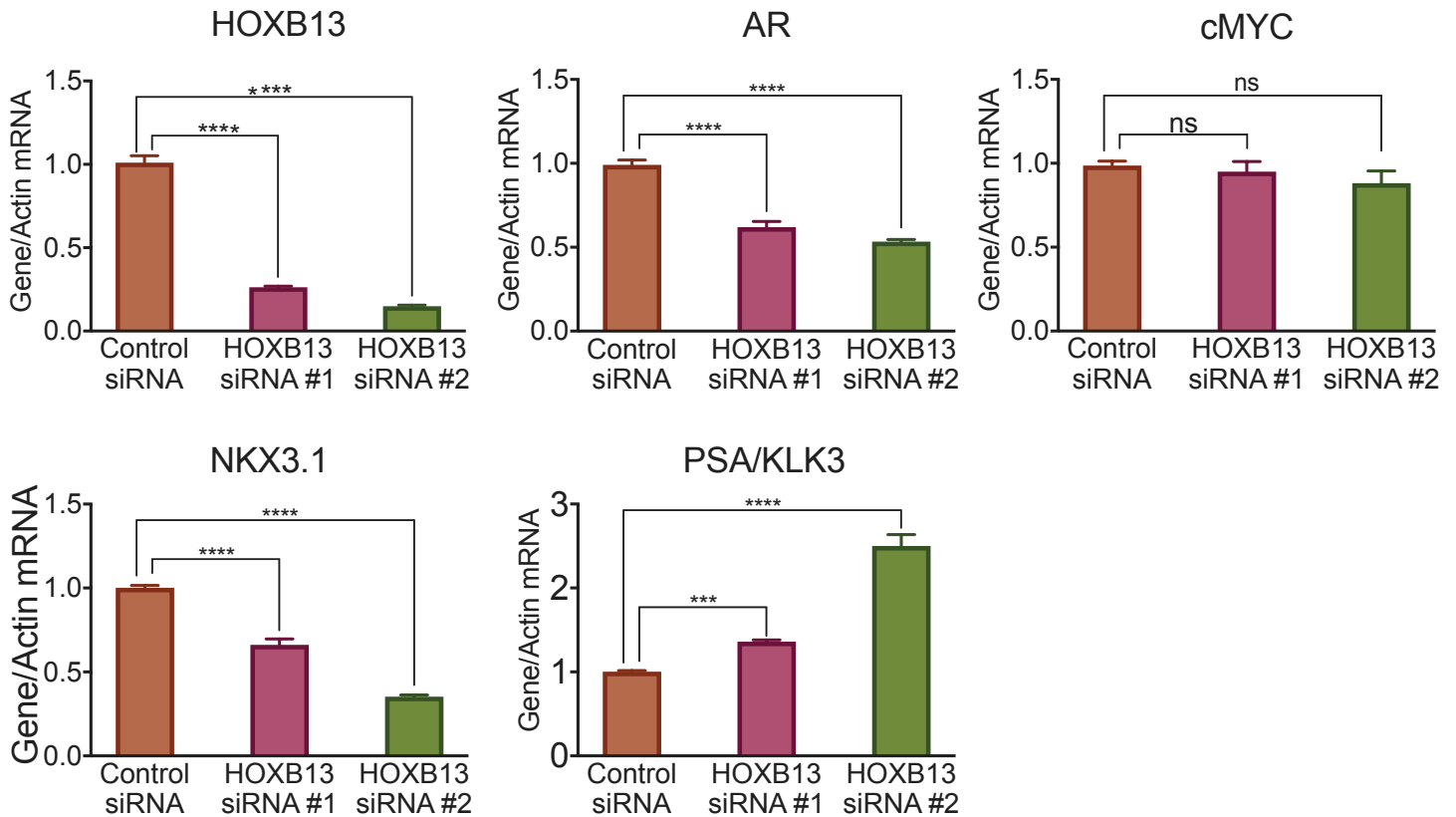
VCaP

a.



b.

C4-2B



Supplementary Figure 3. HOXB13 regulates expression of AR/AR-V7 in metastatic PCs

(a) VCaP cells were transfected control or HOXB13 siRNA and grown in charcoal-stripped androgen-depleted media. Total RNA was isolated, followed by qRT-PCR with HOXB13, AR, AR-V7 and AR target genes (PSA, TMPRSS2 and NKX3.1) primers. **(b)** C4-2B cells were transfected with control or HOXB13 siRNAs (#1 is individual and #2 is pooled). Total RNA was isolated, followed by qRT-PCR with HOXB13, c-MYC, AR, and AR target genes (PSA and NKX3.1) primers. Data are represented as mean \pm SEM and normalized to actin in (a-b). ****p<0.0001, ***p<0.0005, ns: not significant.

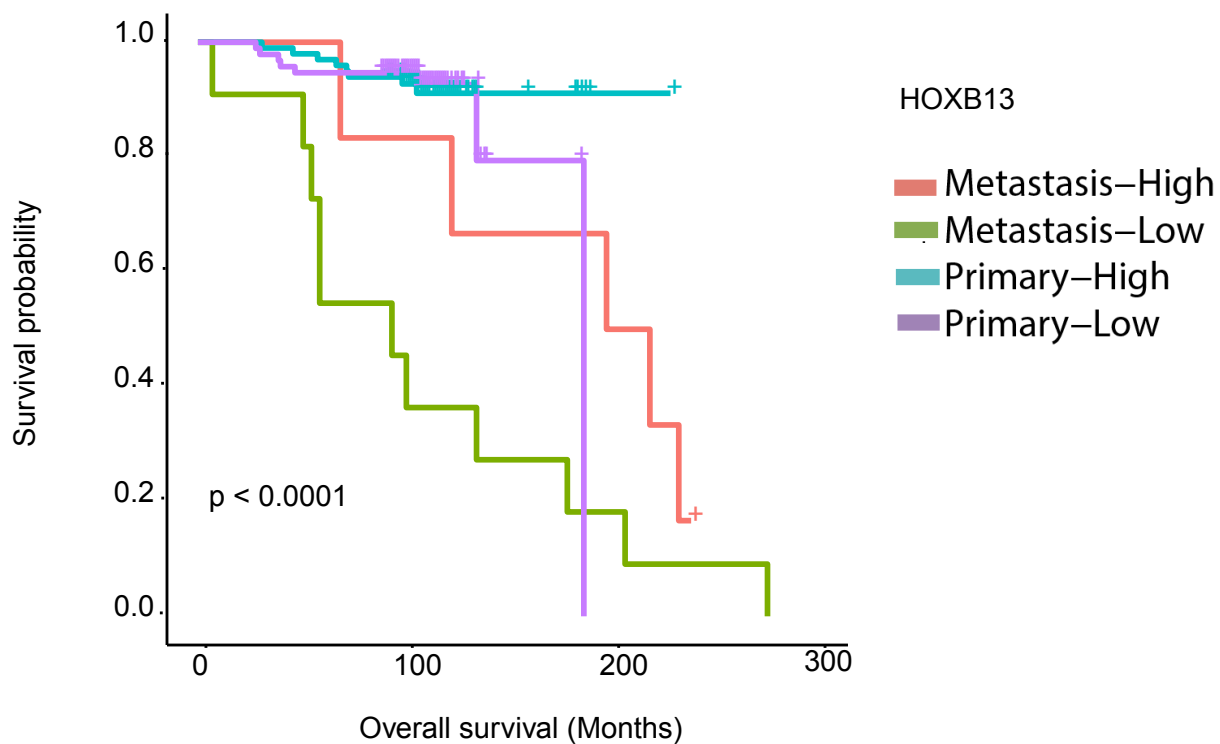
Supplementary Table 1. Association between baseline clinical characteristics with patients disease type for MSKCC data set.

<i>Variable</i>	<i>Metastatic N=19</i>	<i>Primary N=131</i>	<i>P-value</i>
Age at diagnosis (years)			0.15
< 64 Years	12 (63.2%)	103 (78.6%)	
>= 64 Year	7 (36.8%)	28 (21.4%)	
Gleason Score			
5-6	--	41 (31.5%)	
7	--	74 (56.9%)	
8-10	--	15 (11.5%)	
Missing	--	1	
Clinical Tumor Stage			
T1C	--	75 (57.3%)	
T2,T2A,T2B,T2C	--	51 (38.9%)	
T3,T3A,T3B,T3C	--	5 (3.8%)	
Lymph node involvement			<0.001
Abnormal_N1	10 (76.9%)	6 (5.6%)	
Normal_N0	3 (23.1%)	102 (94.4%)	
Missing	6	23	
Margin Status			
Negative	--	100 (76.3%)	
Positive	--	31 (23.7%)	
PSA at Diagnosis			<0.001
< 10	7 (41.2%)	108 (83.1%)	
>= 10	10 (58.8%)	22 (16.9%)	
Missing	2	1	

****P-values for categorical variables calculated using the Fisher's Exact method***

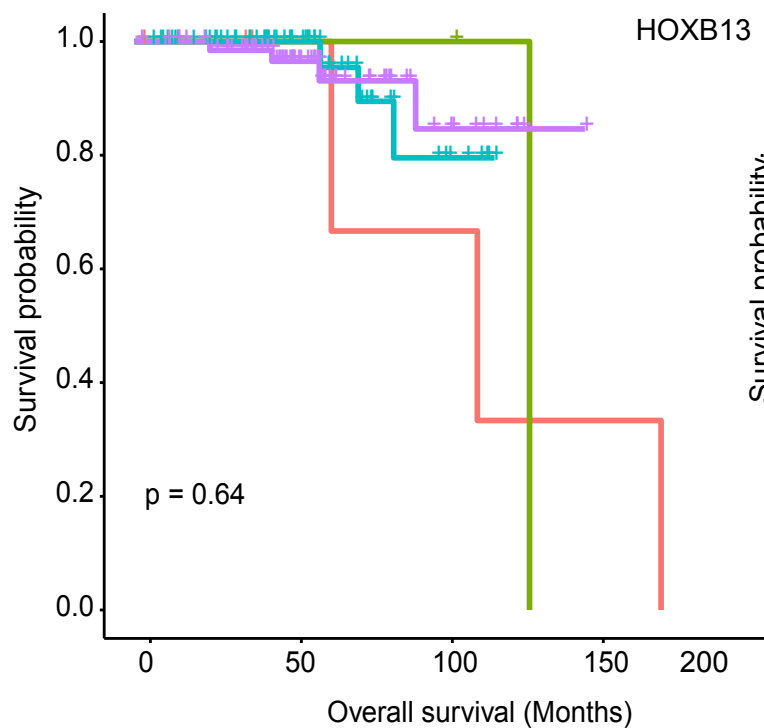
MOFFITT-TCC Data set

a



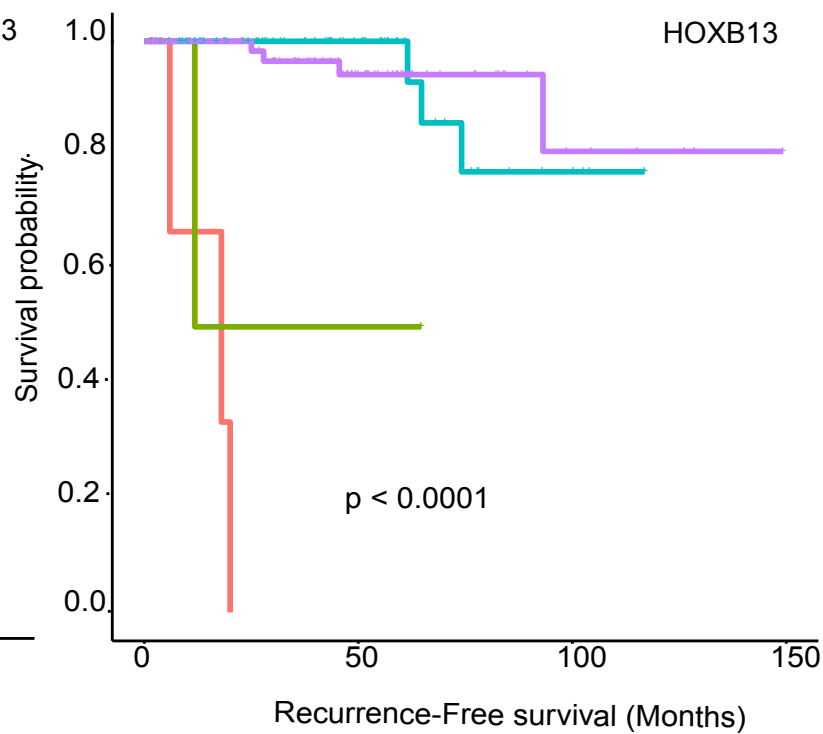
b

MSKCC



c

MSKCC

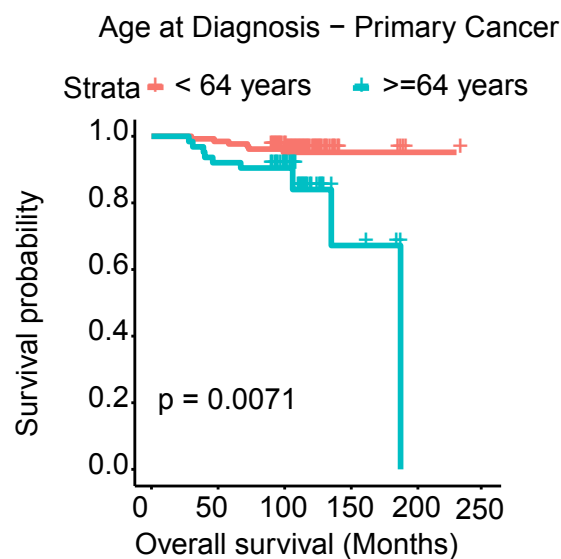


Supplementary Figure 4. Kaplan-Meier overall survival analysis evaluating *HOXB13* gene expression in human prostate cancers

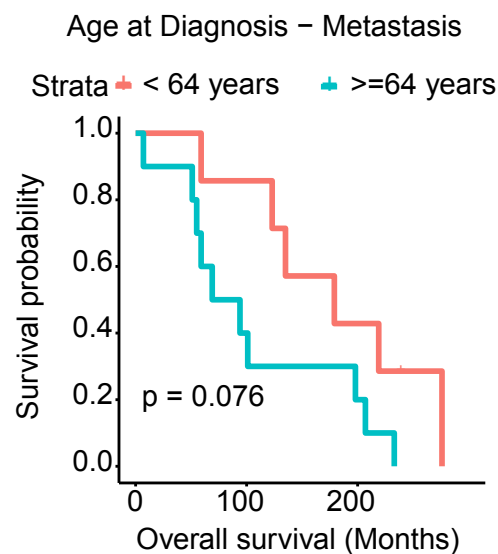
(a) Moffitt TCC data set—overall log rank survival ($p<0.0001$) and **(b)** MSKCC data set—overall log rank survival ($p=0.64$) and **(c)** MSKCC data set—Recurrence Free Survival ($p<0.0001$).

Clinical parameters – Moffitt TCC Data Set

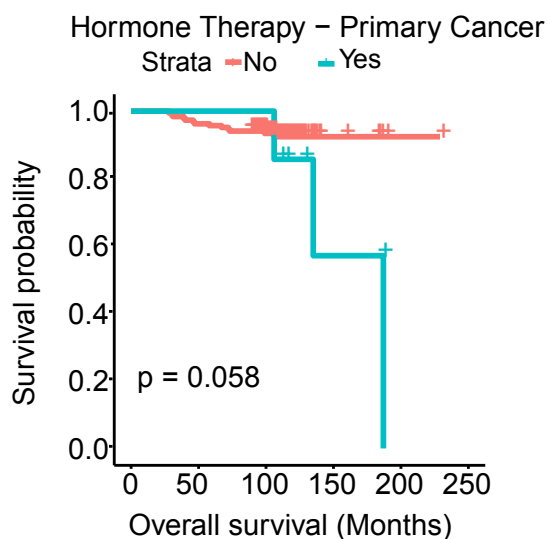
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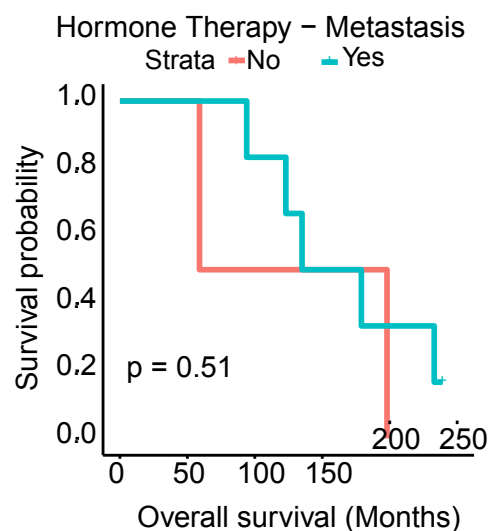
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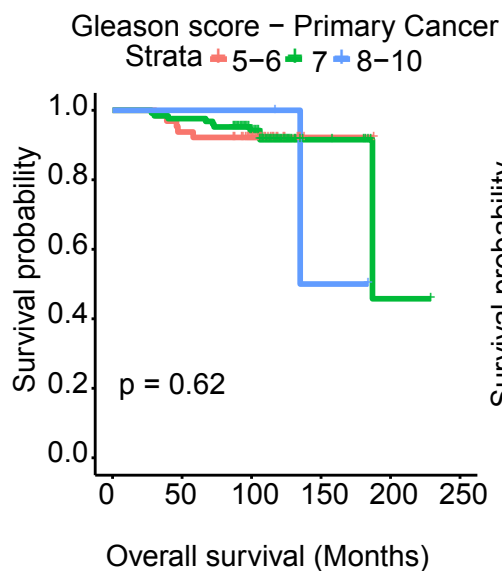
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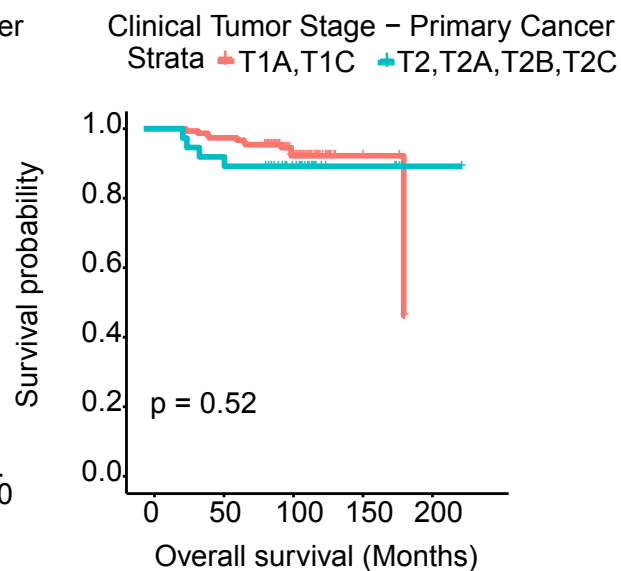
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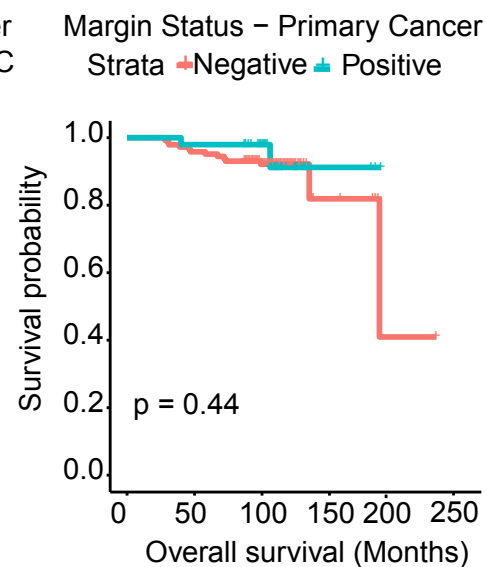
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f



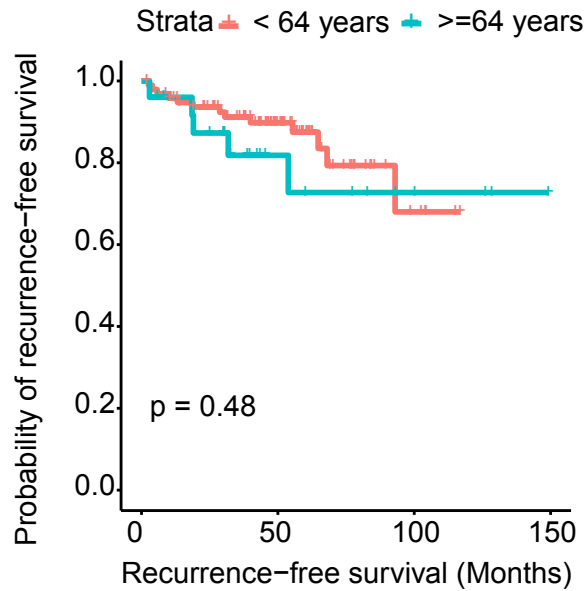
g



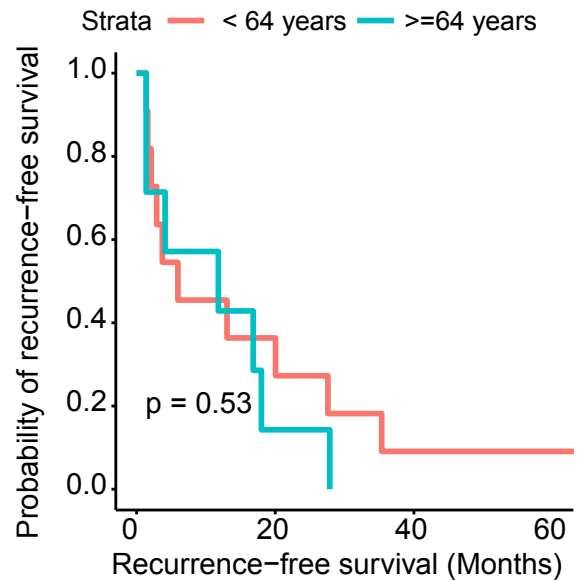
Supplementary Figure 5. Survival analysis of Moffitt TCC data set-Clinical parameters.

(a-g) Kaplan-Meier overall survival curves based on categorized gene expression levels for HOTPAM9 genes in the Moffitt TCC data set. The following parameters were considered: age at diagnosis for primary and metastatic prostate cancer, hormone therapy for primary and metastatic prostate cancer, Gleason score, clinical tumor stage and margin status for primary disease. *p* values were generated by the Log-rank test and univariate Cox proportional hazards regression models.

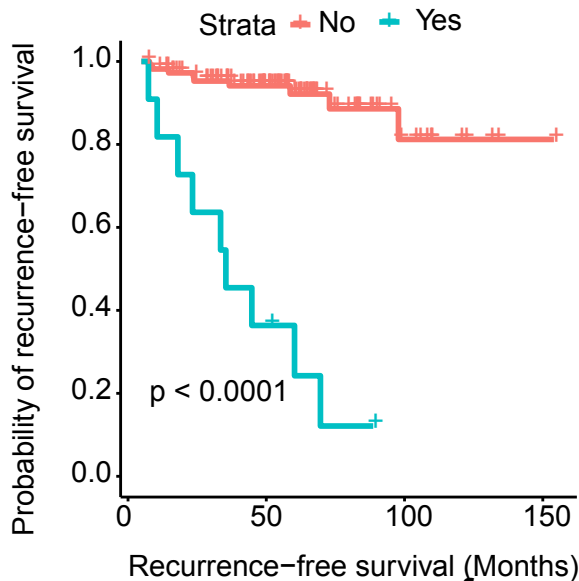
a Age at Diagnosis – Primary Cancer



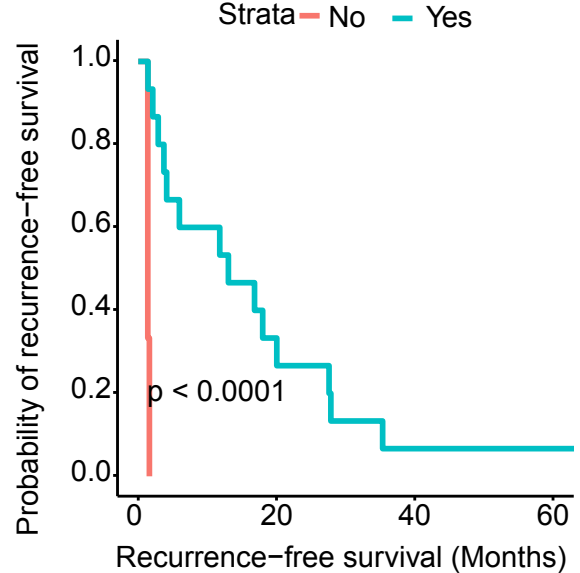
b Age at Diagnosis – Metastasis Cancer



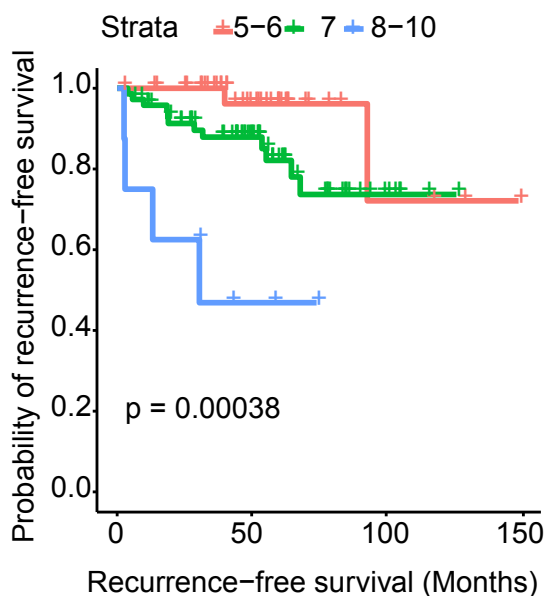
c Hormone Therapy – Primary Cancer



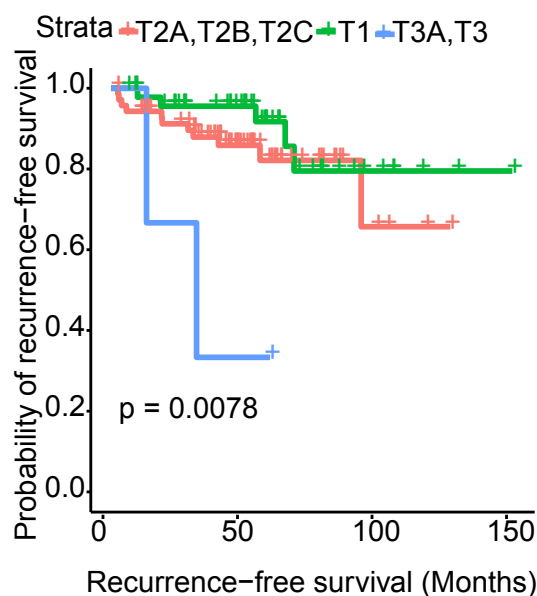
d Hormone Therapy – Metastasis Cancer



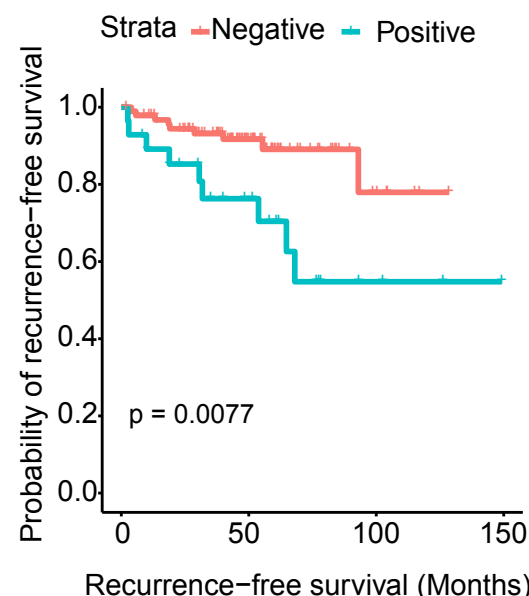
e Gleason score – Primary Cancer



f Clinical Tumor Stage – Primary Cancer



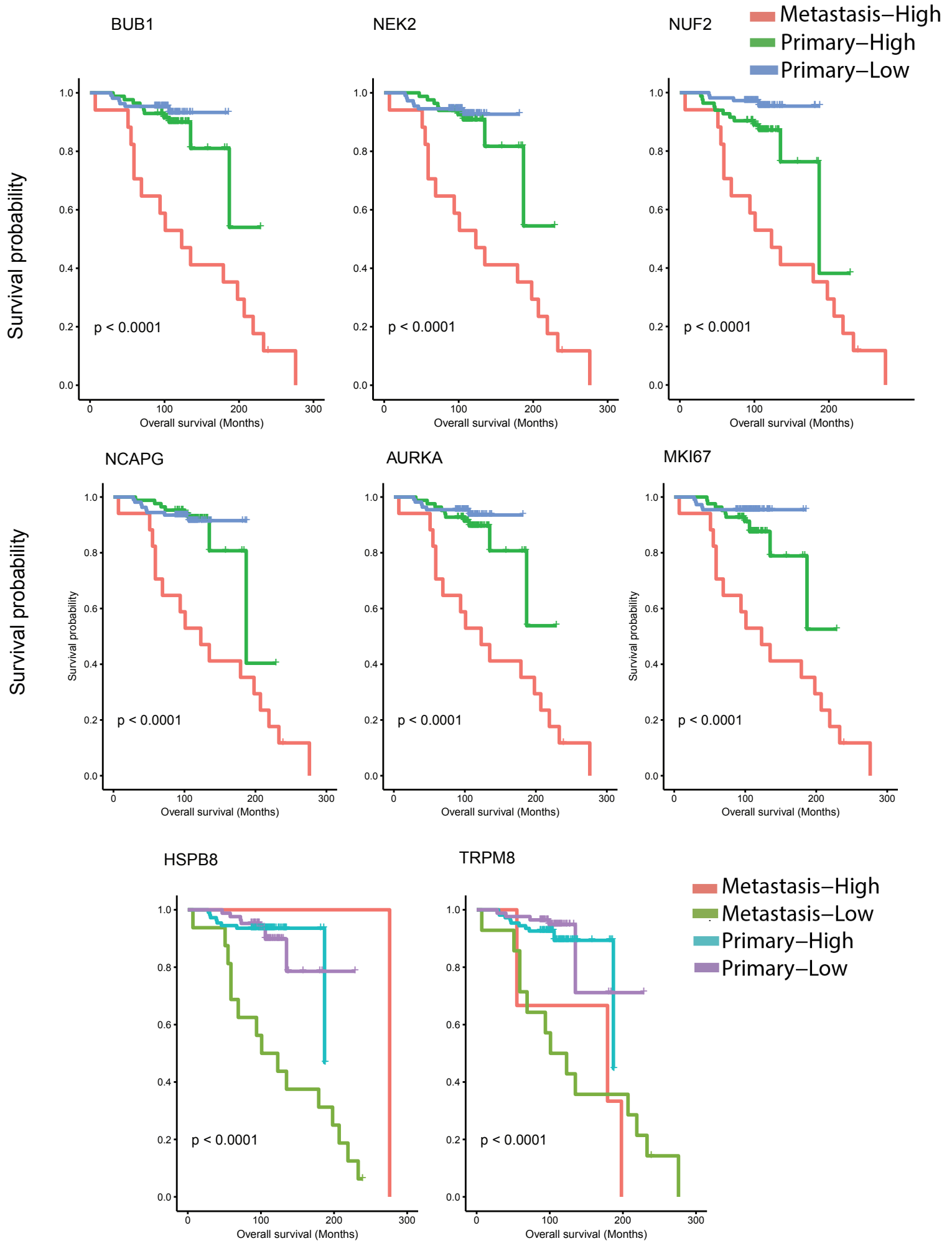
g Margin Status – Primary Cancer



Supplementary Figure 6. Kaplan-Meier Recurrence Free Survival (RFS) analysis of MSKCC data set - clinical parameters.

(a-g) Kaplan-Meier RFS survival curves based on categorized gene expression levels for HOTPAM9 genes in the MSKCC data set. The following parameters were considered: age at diagnosis for primary and metastatic prostate cancer, hormone therapy for primary and metastatic prostate cancer, Gleason score, clinical tumor stage and margin status for primary disease. *p* values were generated by the log-rank test.

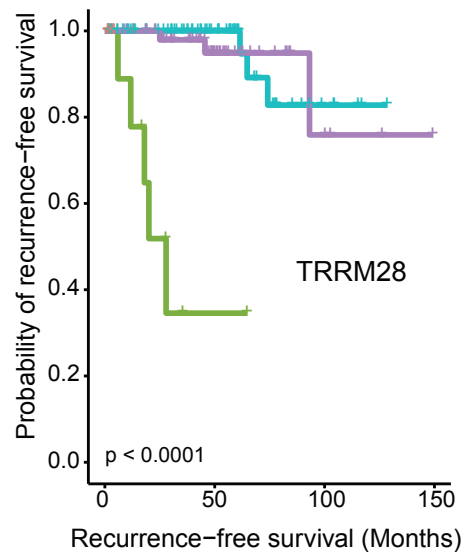
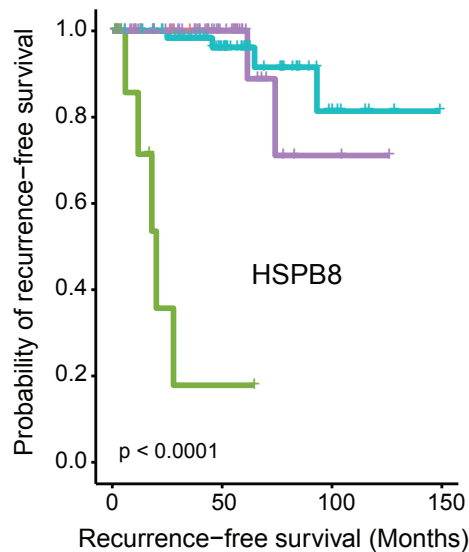
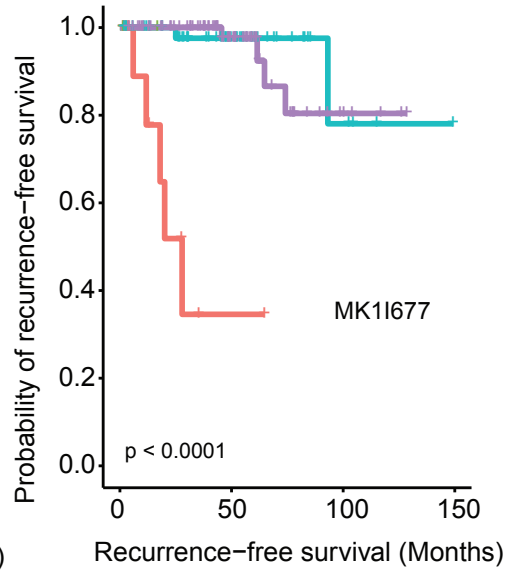
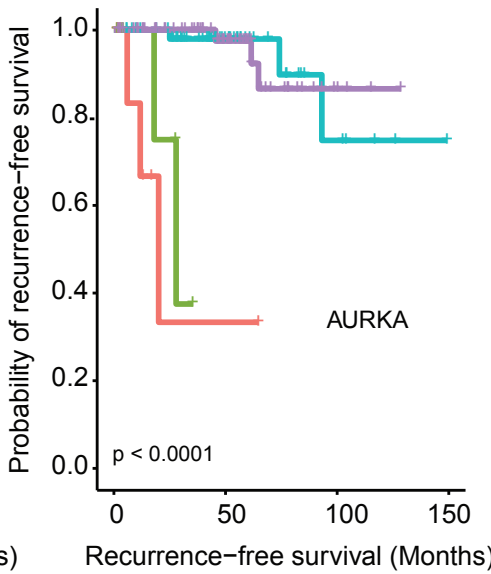
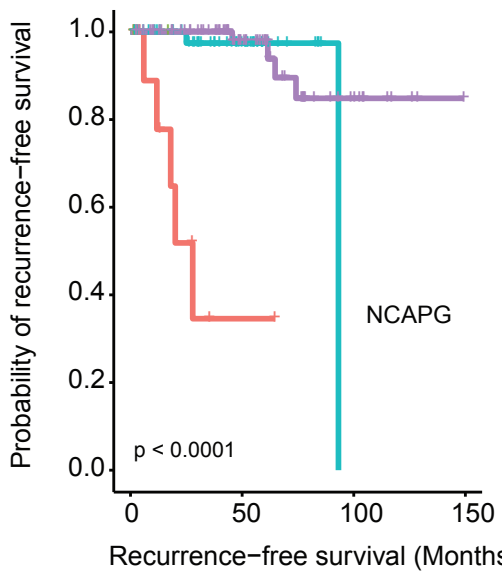
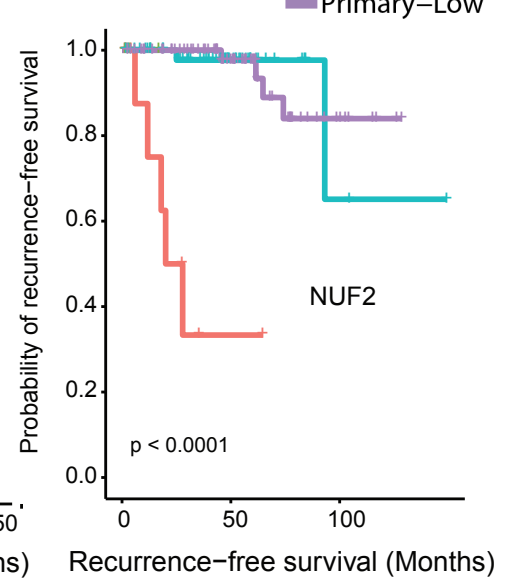
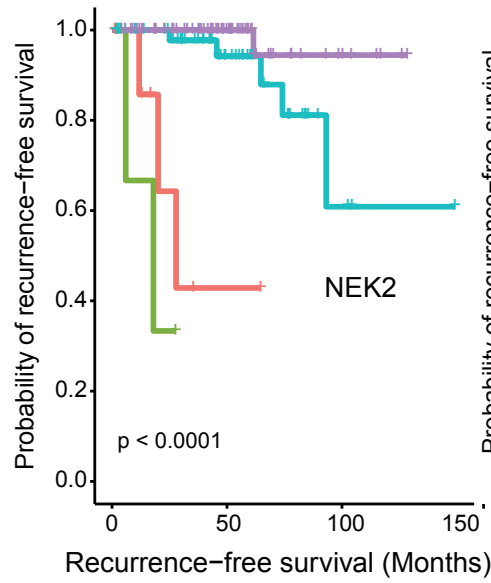
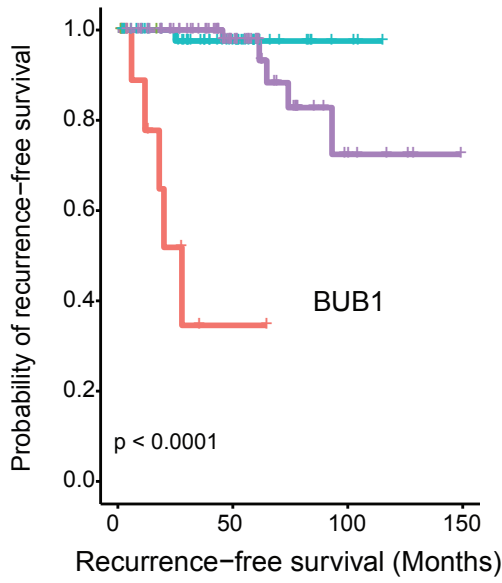
Survival analysis: Moffitt TCC Data set – HOTPAM9 genes



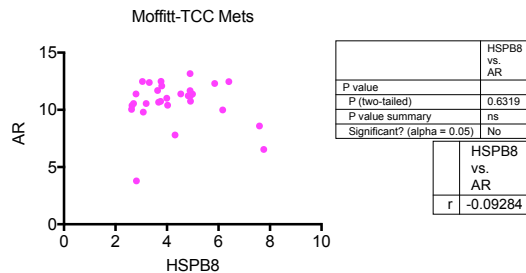
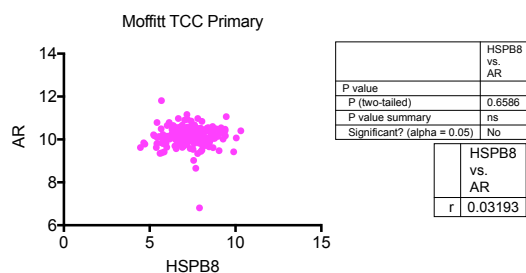
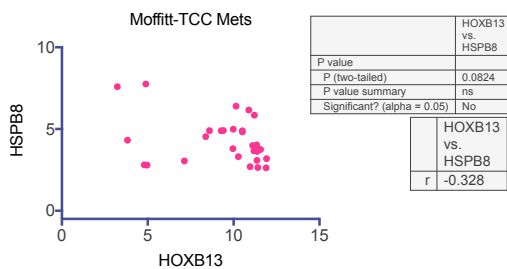
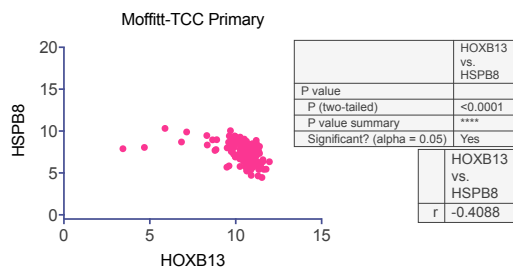
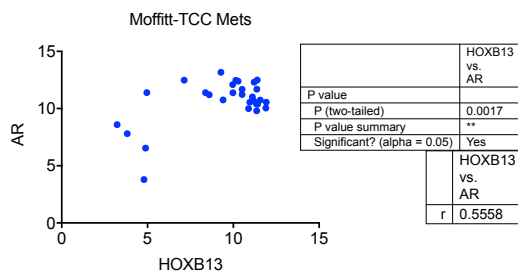
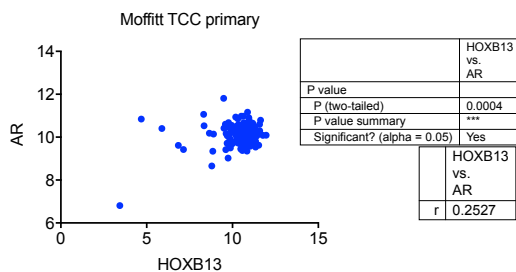
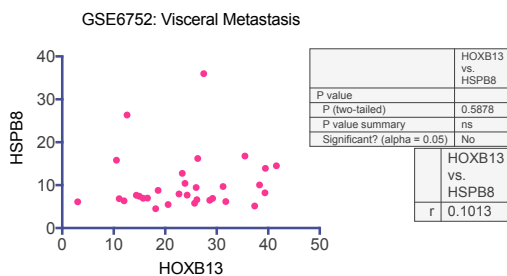
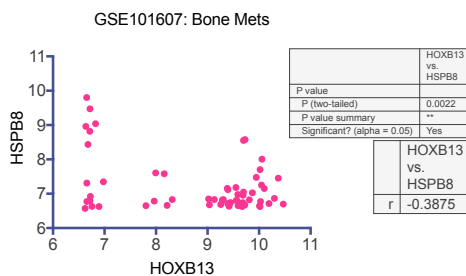
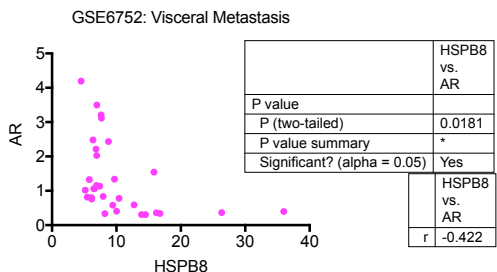
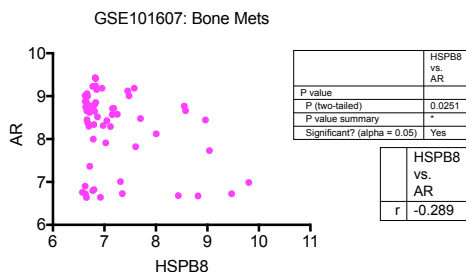
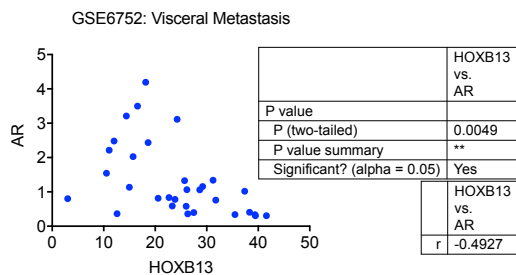
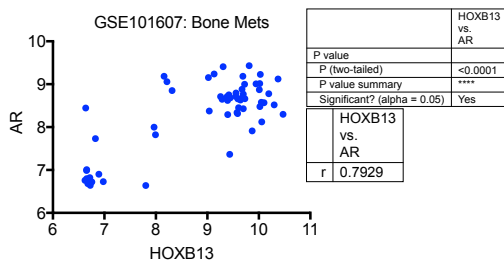
Supplementary Figure 7. Kaplan-Meier overall survival analysis of HOTPAM9 gene set in Moffitt TCC data Set. Kaplan-Meier overall survival curves based on categorized gene expression levels of HOTPAM9 genes in Moffitt TCC data set. *p* values were generated by the log-rank test.

MSKCC Survival Analysis

- Metastasis-High
- Metastasis-Low
- Primary-High
- Primary-Low



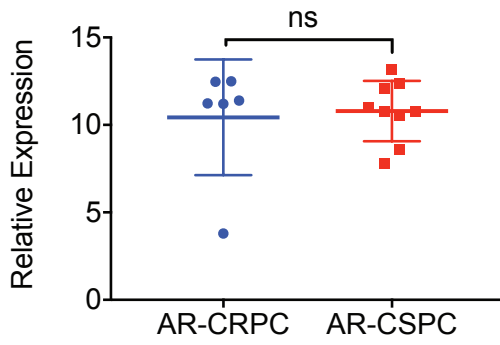
Supplementary Figure 8. Survival analysis evaluating HOTPAM9 gene expression in MSKCC data set. Kaplan-Meier Recurrence Free survival curves based on categorized gene expression levels of HOTPAM9 genes in MSKCC data set. *p* values were generated by the log-rank test.



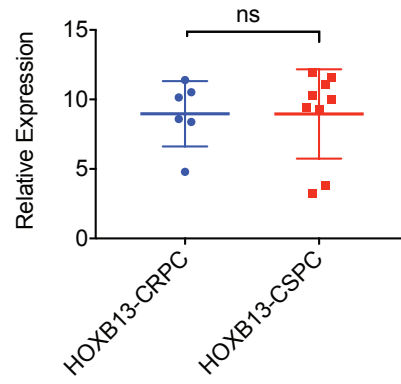
Supplementary Figure 9. Low levels of HSPB8 mRNA expression negatively correlate with increased HOXB13 expression in PCs

Pearson correlation analysis was performed on multiple metastatic PC data sets (GSE101607, GSE6752, GSE67980 and Moffit TCC data sets) to examine the correlation between AR, HOXB13 and HSPB8 expression in PCs metastasized to the bone, lymph node, lung, liver and other organs. Pearson regression co-efficient (r) values and p values are shown in the tables.

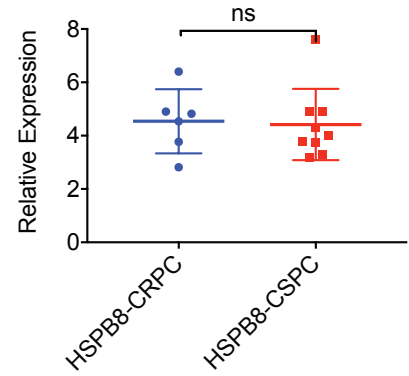
Moffitt TCC-Metastasis



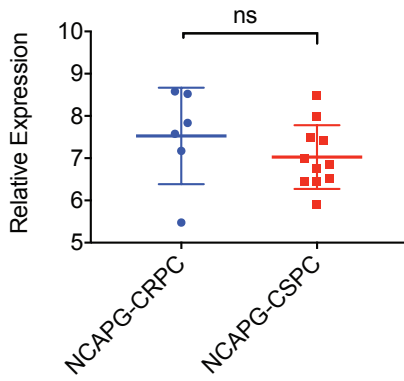
Moffitt TCC-Metastasis



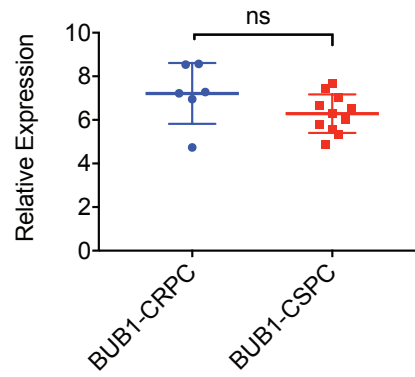
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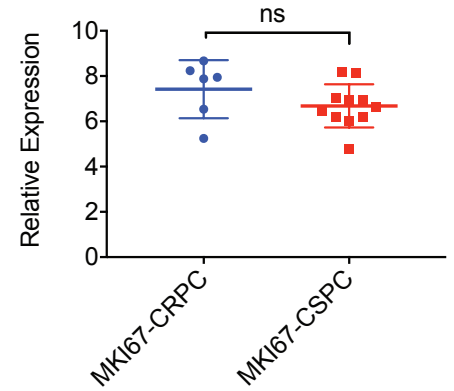
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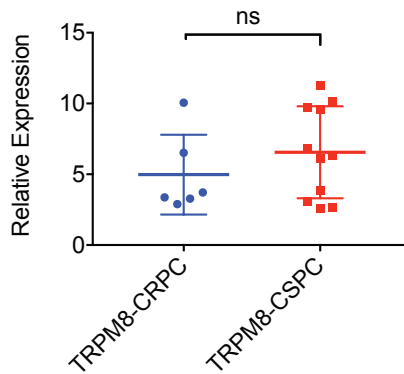
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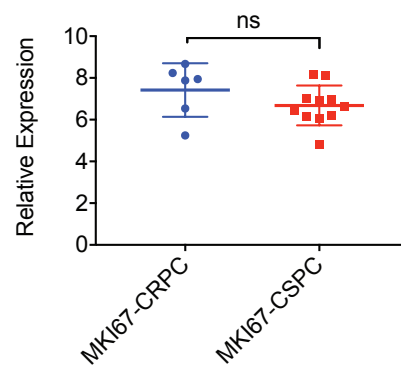
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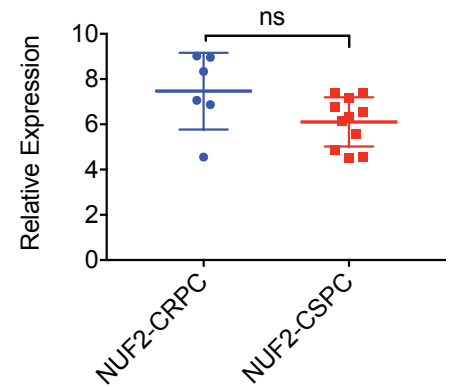
Moffitt TCC-Metastasis



Moffitt TCC-Metastasis



Moffitt TCC-Metastasis



Supplementary Figure 10. Expression of HOTPAM9 genes in metastatic PC samples pre- and post-anti-androgen therapies. Relative expression of HOTPAM9 genes pre- and post-castration treatment in the Moffitt TCC data set. ns: not significant. The metastatic samples were obtained from multiple sites (lung, liver, bone, lymph nodes and soft tissue), while primary samples were obtained from anterior/posterior/apex/mid/base of prostate.

Uncropped images of Western blots presented in Figure 3a, 8a, 8b

Figure 3a

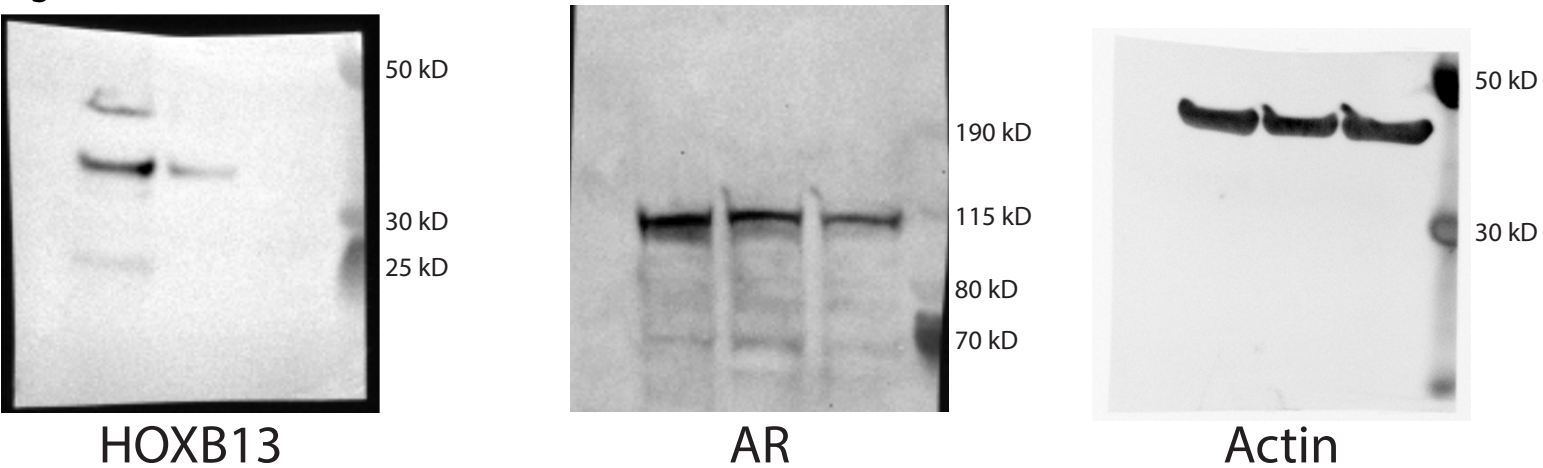
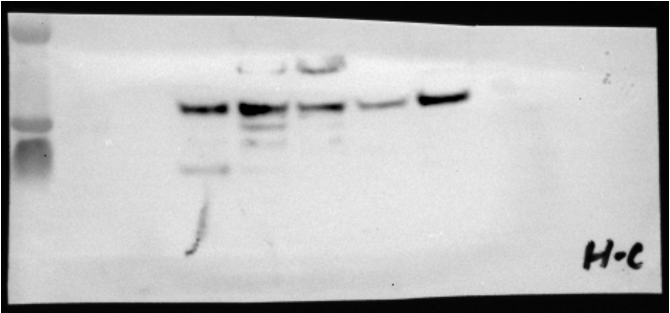
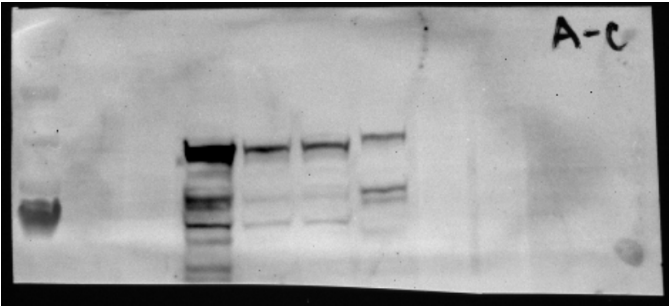


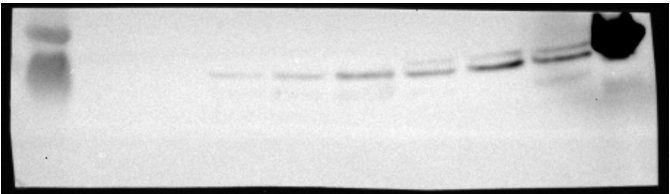
Figure 8a



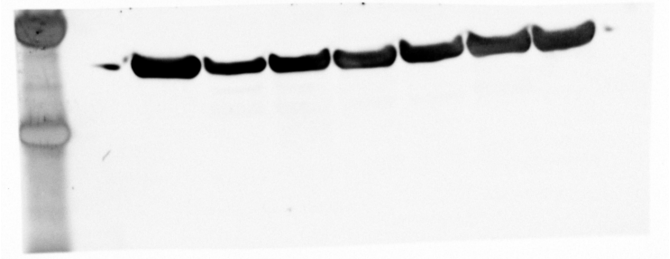
HOXB13



AR

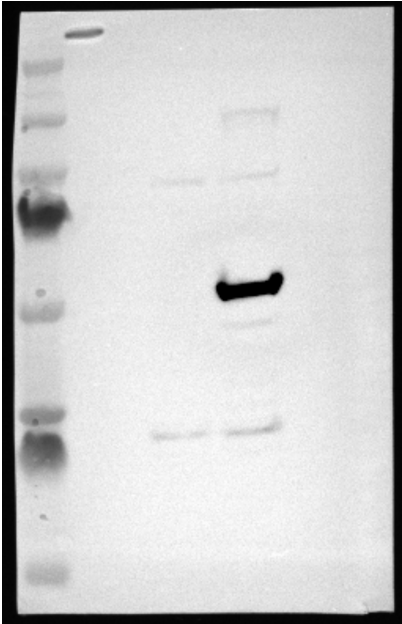


HSPB8

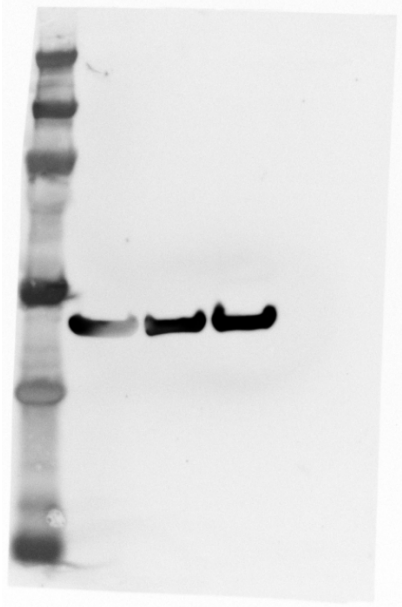


Actin

Figure 8b



HSPB8



Actin