

# Supplementary Materials: Molecular and clinical relevance of *ZBTB38* expression levels in prostate cancer.

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**Table S1.** *ZBTB38* expression levels and *ETS* transcription factor gene fusions in localised prostate tumours.

Characteristics	Taylor et al. 2010 (GSE21032)				TCGA. 2015 (cBioPortal)				Ross-Adams et al. 2015 (GSE70770)			
	<i>ZBTB38</i> expression (N)		Chi2	<i>p</i> -value	<i>ZBTB38</i> expression (N)		Chi2	<i>p</i> -value	<i>ZBTB38</i> expression (N)		Chi2	<i>p</i> -value
	Low	High			Low	High			Low	High		
<b>ERG fusion (by gene expression)</b>												
<i>No fusion</i>	34	30			-	-			29	26		
<i>Fusion</i>	30	35	0.626	0.428	-	-	-	-	24	28	0.462	0.496
<b>ERG fusion (by CGH*)</b>												
<i>No fusion</i>	45	33			-	-			-	-		
<i>Fusion</i>	13	17			-	-			-	-		
<i>Flat</i>	6	15	1.796	0.18	-	-	-	-	-	-	-	-
<b>ERG fusion (by exon sequencing)</b>												
<i>No fusion</i>	-	-			57	72			-	-		
<i>Fusion</i>	-	-	-	-	88	72	3.34	0.067	-	-	-	-
<b>ETV1 fusion (by exon sequencing)</b>												
<i>No fusion</i>	-	-			141	140			-	-		
<i>Fusion</i>	-	-	-	-	4	5	0.236	0.626	-	-	-	-
<b>ETV4 fusion (by exon sequencing)</b>												
<i>No fusion</i>	-	-			135	138			-	-		
<i>Fusion</i>	-	-	-	-	10	7	0.114	0.734	-	-	-	-

*N*, number of patients per class. Significant *p*-value ( $p < 0.05$ ) are highlighted in bold. \* CGH: Comparative Genomic Hybridization.

**Table S2.** Correlation between *ZBTB38* expression levels and recurrent copy number aberrations (CNA) in localised prostate tumours.

Characteristics	TCGA. 2015 (cBioPortal)			
	<i>ZBTB38</i> Expression (N)		Chi2	<i>p</i> -Value
	Low	High		
<b>BRCA1 CNA</b>				
<i>Diploid</i>	133	126		
<i>Loss</i>	12	19	1.769	0.183
<b>BRCA2 CNA</b>				
<i>Diploid</i>	116	126		
<i>Loss</i>	29	19	2.496	0.114
<b>CDK12 CNA</b>				
<i>Diploid</i>	137	138		
<i>Loss</i>	8	7	0.07	0.79
<b>CHD1 CNA</b>				
<i>Diploid</i>	113	132		
<i>Loss</i>	32	13	9.495	<b>0.002</b>
<b>FAM175A CNA</b>				
<i>Diploid</i>	142	143		
<i>Loss</i>	3	2	0.203	0.651
<b>FANCC CNA</b>				
<i>Diploid</i>	125	140		
<i>Gain</i>	16	4		

<i>Loss</i>	4	1	9.849	<b>0.007</b>
<b>FANCD2 CNA</b>				
<i>Diploid</i>	134	130		
<i>Loss</i>	11	15	0.067	0.410
<b>PTEN CNA</b>				
<i>Diploid</i>	105	101		
<i>Loss</i>	40	44	0.268	0.604
<b>RAD51C CNA</b>				
<i>Diploid</i>	136	141		
<i>Loss</i>	5	4		
<i>Gain</i>	4	0	4.201	0.122
<b>RB1 CNA</b>				
<i>Diploid</i>	90	118		
<i>Loss</i>	55	27	13.33	<b>0.0002</b>
<b>SPOPL CNA</b>				
<i>Diploid</i>	115	138		
<i>Loss</i>	30	7	16.388	<b>0.00005</b>
<b>TP53 CNA</b>				
<i>Diploid</i>	98	100		
<i>Loss</i>	47	45	0.063	0.8

*N*, number of patients per class Significant *p*-value ( $p < 0.05$ ) are highlighted in bold.

**Table S3.** Correlation between *ZBTB38* expression levels and recurrent mutations in localised prostate tumours.

Characteristics	TCGA. 2015 (cBioPortal)			Chi2	<i>p</i> -Value
	<i>ZBTB38</i> Expression ( <i>N</i> )				
	Low	High			
<b><i>KMT2C</i></b>					
<i>No mutation</i>	138	140			
<i>Mutation</i>	7	5	0.347	0.555	
<b><i>KMT2D</i></b>					
<i>No mutation</i>	141	138			
<i>Mutation</i>	4	7	0.85	0.356	
<b><i>SPOP</i></b>					
<i>No mutation</i>	121	138			
<i>Mutation</i>	24	7	10.438	<b>0.001</b>	
<b><i>TP53</i></b>					
<i>No mutation</i>	132	138			
<i>Mutation</i>	13	7	1.933	0.164	

Only recurrent mutations (i.e. present in > 10 samples) were evaluated.

*N*, number of patients per class. Significant *p*-value ( $p < 0.05$ ) are highlighted in bold.

**Table S4.** Correlation between *ZBTB38* expression levels and molecular data in localised prostate tumours.

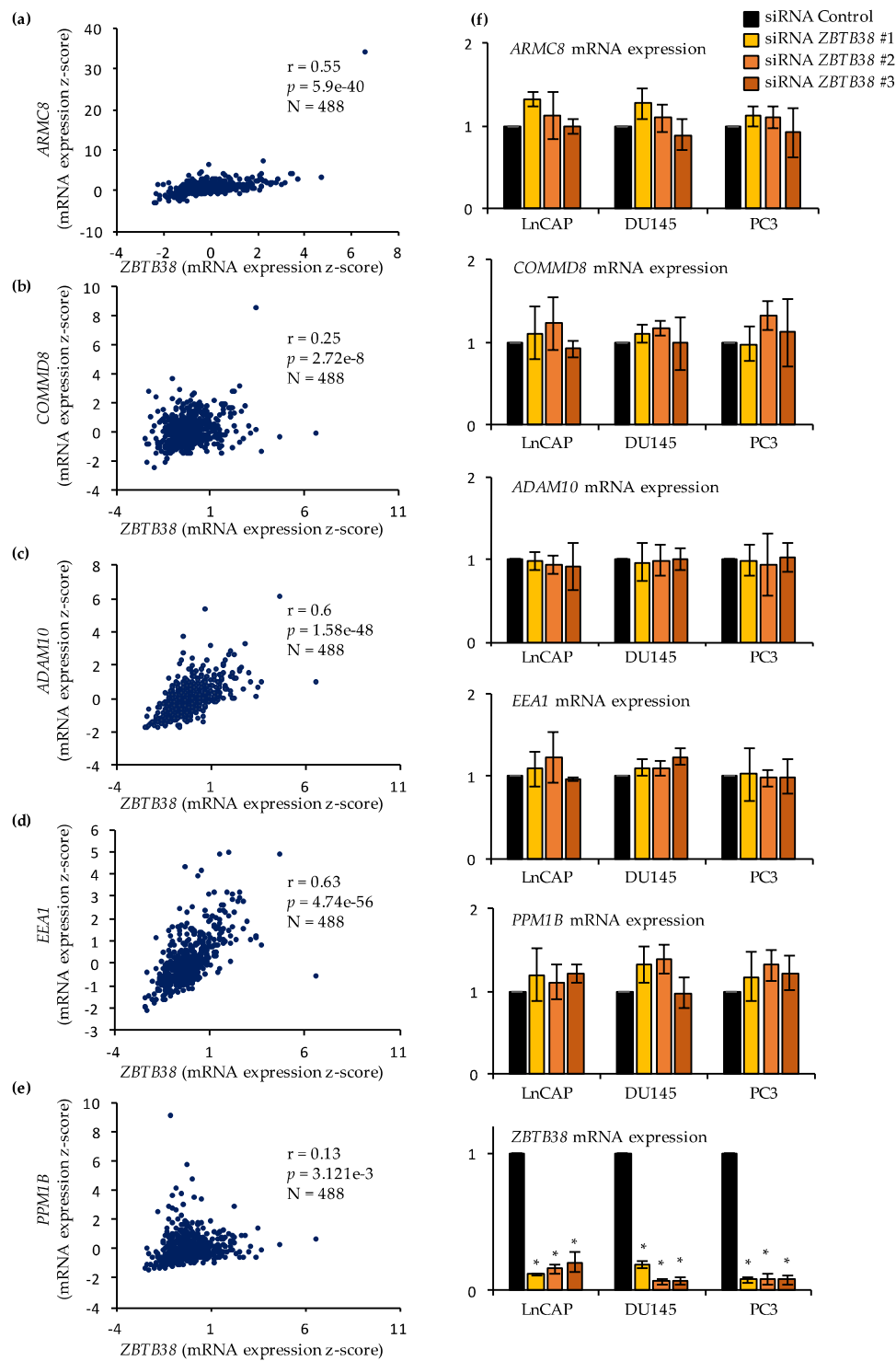
Characteristics	TCGA, 2015 (cBioPortal)			
	ZBTB38 Expression (N)		Chi2	p-value
	Low	High		
<b>RPPA clusters</b>				
Cluster 1	23	39		
Cluster 2	20	24		
Cluster 3	26	13		
Cluster 4	16	19		
Cluster 5	23	14	11.268	<b>0.023</b>
<b>DNA methylation cluster</b>				
Cluster 1	20	14		
Cluster 2 <sup>#</sup>	65	31		
Cluster 3	38	50		
Cluster 4	22	49	25.001	<b>&lt;0.00001</b>
<b>mRNA Cluster</b>				
Cluster 1 <sup>#</sup>	64	37		
Cluster 2	59	38		
Cluster 3	22	70	36.80	<b>&lt;0.00001</b>
<b>Integrative Cluster (iCluster)</b>				
Cluster 1	55	21		
Cluster 2	58	39		
Cluster 3	32	84	42.239	<b>&lt;0.00001</b>

Unsupervised clustering of RPPA, DNA methylation and mRNA data were performed by the TCGA consortium (Ref. 6). #, SPOP mutations are associated with DNA methylation cluster 2 and mRNA cluster 1. N, number of patients per class. Significant *p*-value ( $p < 0.05$ ) are highlighted in bold.

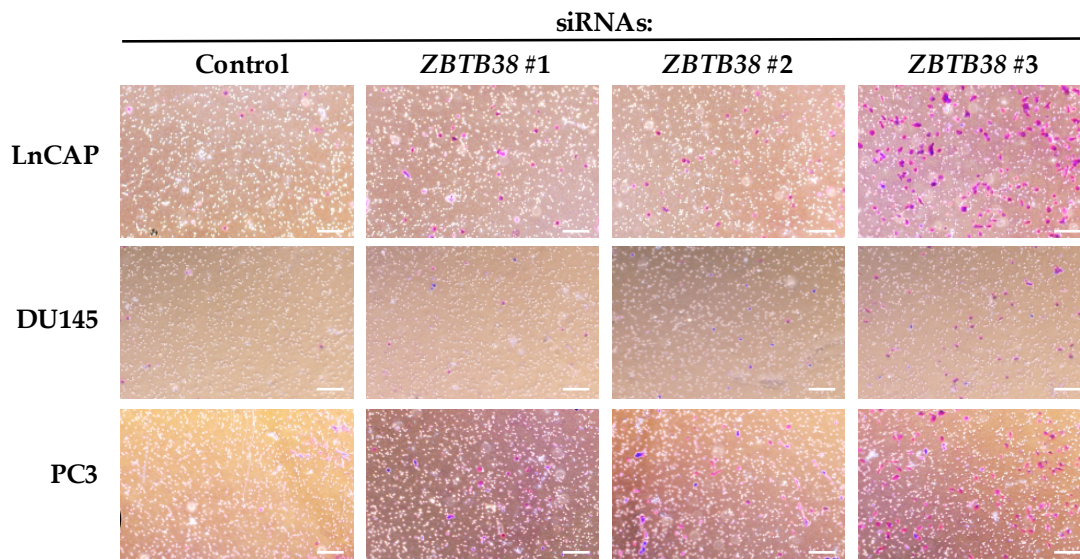
**Table S5.** Correlation between *ZBTB38* expression levels and chromosomal instability in metastatic prostate tumours.

Characteristics	Robinson et al. 2015 (dbGap: phs000915.v1.p1)			Abida et al. 2019 (cBioPortal)			Kumar et al. 2016 (GSE77930)					
	<i>ZBTB38</i> expression (N)		Chi2	<i>p</i> - value	<i>ZBTB38</i> expression (N)		Chi2	<i>p</i> - value	<i>ZBTB38</i> expression (N)		Chi2	<i>p</i> - value
	<i>Low</i>	<i>High</i>			<i>Low</i>	<i>High</i>			<i>Low</i>	<i>High</i>		
<b>Age (years)</b>												
<60	10	12			37	43			63	58		
>60	48	45	0.269	0.603	55	50	0.682	0.408	3	9	3.199	0.073
<b>Fraction genome altered</b>												
0 - 0.1	4	4			7	12			4	3		
0.1 - 0.5	39	34			64	69			35	45		
>0.5	16	21	1.018	0.601	35	24	3.549	0.169	28	19	3.116	0.21
<b>Mutation count</b>												
<50	7	9			34	31			37	40		
50 - 100	36	32			42	42			15	21		
>100	16	18	0.602	0.739	29	32	0.286	0.866	14	6	4.309	0.115

*N*, number of patients per class. Significant *p*-value ( $p < 0.05$ ) are highlighted in bold.



**Figure S1.** Correlation between *ZBTB38* expression and *ARMC8*, *COMMD8*, *ADAM10*, *EE1A* and *PPM1B* expression in prostate cancer; consequences of *ZBTB38* knock-down on gene expression. (a–e) Gene expression levels from TCGA (ref. 13) were plotted to illustrate the correlation between *ZBTB38* expression and: (a) *ARMC8*, (b) *COMMD8*, (c) *ADAM10*, (d) *EE1A* and (e) *PPM1B*. The  $r$  value indicates Pearson coefficients and the corresponding  $p$ -value are indicated.  $N$ , number of tumours analysed. (f) qRT-PCR analysis of *ARMC8*, *COMMD8*, *ADAM10*, *EE1A*, *PPM1B* and *ZBTB38* in LnCAP, DU145 and PC3 cells transfected with *ZBTB38* and control siRNAs ( $n = 3$ ). \*,  $p < 0.05$  (vs. Control).



**Figure S2.** Representative images of transwell migration assays after transfection with control or *ZBTB38* siRNAs in LnCAP, DU145 and PC3 cancer cells. Cell numbers per field are presented in Figure 5c.



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