

Supplementary Materials

Supplemental Table 1. Pathological and clinical variables of training and validation cohorts

		Training (n, %)	Validation (n, %)
All patients		410	272
Age of patients at surgery, yr			
	Mean	62.6	59.7
	Median	63	61
	Range (min-max)	45-77	44-72
	Missing	4 (1.0%)	0 (0.0%)
PSA (ng/ml)			
	Mean	9	6.7
	Median	7.2	5.8
	Range (min-max)	0.2-66.0	0.45-33.0
	Missing	7 (1.7%)	5 (1.8%)
Recurrence			
	Yes	143 (34.9%)	54 (19.9%)
	No	260 (63.4%)	218 (80.1%)
	Missing	7 (1.7%)	0 (0.0%)
Follow-up time (yrs)			
	Mean	6.7	5
	Median	5.7	4.9
	Range (min-max)	0.0-21.8	0.09-13.3
	Missing	6 (1.5%)	34 (12.5%)
Prostatectomy Grade Groups			
	GG1	147 (35.8%)	70 (25.7%)
	GG2	132 (32.2%)	170 (62.5%)
	≥GG3	122 (29.8%)	32 (11.8%)
	Missing	9 (2.2%)	0 (0.0%)
pStage			
	≤pT2	295 (72.0%)	214 (78.7%)
	pT3a	61 (14.9%)	50 (18.4%)
	pT3b	48 (11.7%)	8 (2.9%)
	Missing	6 (1.4%)	0 (0.0%)
SVI			
	Yes	30	8
	No	376	264
	Missing	4	0
EPE			
	Yes	104	58
	No	302	214

	Missing	4	0
SM	Yes	136	37
	No	265	235
	Missing	9	0
ERG Fusion	Yes	192	171
	No	181	79
	Missing	37	22
PTEN Loss	Yes	98	54
	No	279	203
	Missing	33	15

PSA=prostate specific antigen, GG=grade group, pStage=pathological stage, SVI=Seminal Vesicle Invasion, EPE=Extra-prostatic Extension, SM=Surgical Margin

Supplemental Table 2. Immunohistochemical staining conditions and imaging techniques

	CHUM cohort		KHSC and Brazil cohorts	
	PTEN	ERG	PTEN	ERG
Staining platform	Benchmark XT*	Benchmark XT*	Discovery XT†	Discovery XT†
Antibody clone ID	SP218	9FY	D4.3 XP	EPR3864
Antibody species	Rabbit mAb	Mouse mAb	Rabbit mAb	Rabbit mAb
Vendor for antibody	Spring Bioscience	Biocare Medical	Cell Signaling Technologies	Epitomics
Antibody dilution	1 : 50	1 : 25	1 : 100	1 : 50
Pre-treatment solution	CC1‡	CC1‡	CC1‡	CC1‡
Antigen retrieval time	60 min	90 min	60 min	60 min
Detection kit	UltraView Universal DAB§	UltraView Universal DAB§	DISCOVERY OmniMap	DISCOVERY OmniMap
Slides scanner	Olympus	Olympus	Aperio (Leica Biosystems)	Aperio (Leica Biosystems)
Digital scoring software	HALO (Indica Labs)	HALO (Indica Labs)	HALO (Indica Labs)	HALO (Indica Labs)

*Benchmark XT, Ventana Medical System, Inc., Tucson, AZ, USA

†Discovery XT, Ventana Medical System, Inc., Tucson, AZ, USA

‡CC1, Cell Conditioning 1, Ventana Medical System Inc., Catalog #950-124

§UltraView Universal DAB, Ventana Medical Systems, Inc.

||DISCOVERY OmniMap, Ventana Medical Systems, Inc.

Supplemental Table 3a. Association of PTEN and ERG immunohistochemistry status with clinicopathologic factors in the training cohort

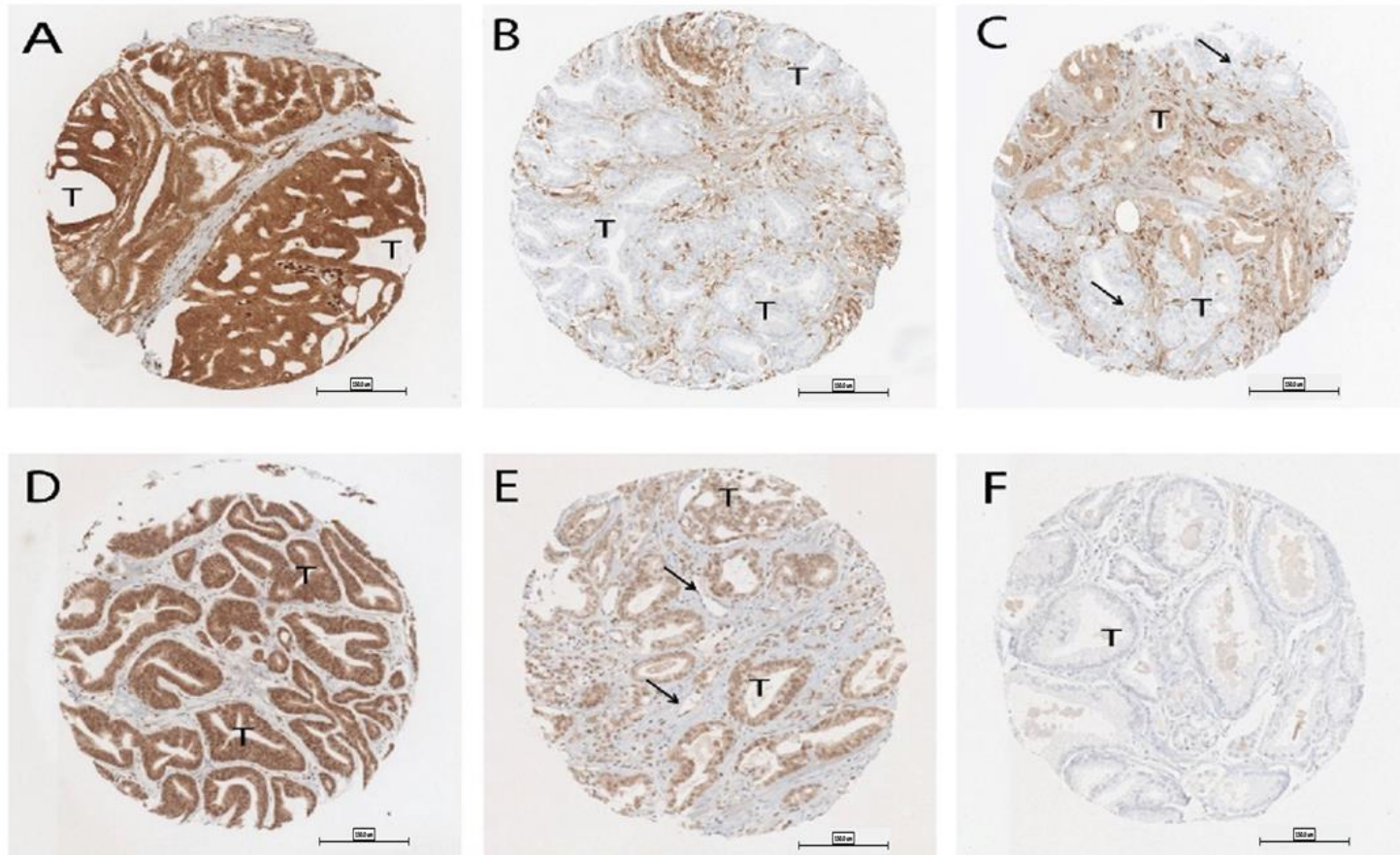
	PTEN status		p-value	ERG fusion		p-value
	Any loss	Intact		No	Yes	
Prostatectomy						
Grade Groups						
GG1	28 (19.7%)	114 (80.3%)	0.09	66 (47.5%)	73 (52.5%)	0.60
GG2	34 (27.2%)	91 (72.8%)		59 (47.6%)	65 (52.4%)	
≥GG3	32 (31.7%)	69 (68.3%)		54 (53.5%)	47 (46.5%)	
Total	94 (25.5%)	274 (74.5%)		179 (49.2%)	185 (50.8%)	
Missing	42 (11.4%)			46 (12.6%)		
Time to Recurrence						
No	47 (20.0%)	188 (80.0%)	< 0.001*	111 (47.6%)	122 (52.4%)	0.49*
Yes	49 (36.3%)	86 (63.7%)		68 (51.1%)	65 (48.9%)	
Total	96 (25.9%)	274 (74.1%)		179 (48.9%)	187 (51.1%)	
Missing	38 (10.3%)			44 (12.0%)		
pStage						
T2	59 (21.6%)	214 (78.4%)	0.009	137 (50.9%)	132 (49.1%)	0.35
T3a	22 (37.3%)	37 (62.7%)		24 (40.7%)	35 (59.3%)	
T3b	15 (37.5%)	25 (62.5%)		19 (47.5%)	21 (52.5%)	
Total	96 (25.8%)	276 (74.2%)		180 (48.9%)	188 (51.1%)	
Missing	38 (10.2%)			42 (11.4%)		
PTEN Loss						
No				37 (37.8%)	61 (62.2%)	0.01†
Yes				144 (52.4%)	131 (47.6%)	
Total				181 (48.5%)	192 (51.5%)	
Missing				37 (9.9%)		
SVI						
No	89(25.4%)	261 (74.6%)	0.62†	167 (48.3%)	179 (51.7%)	0.52†
Yes	7 (30.4%)	16 (69.6%)		13 (56.5%)	10 (43.5%)	
Total	96 (25.7%)	277 (74.3%)		180 (48.8%)	189 (51.2%)	
Missing	37 (9%)			41 (10%)		
EPE						
No	59 (21.5%)	215 (78.5%)	0.003†	138 (51.1%)	132 (48.9%)	0.16†
Yes	37 (37.4%)	62 (62.6%)		42 (42.4%)	57 (57.6%)	
Total	96 (25.7%)	277 (74.3%)		180 (48.8%)	189 (51.2%)	
Missing	37 (9%)			41 (10%)		
SM						
No	58 (76.4%)	188 (76.4%)	0.17†	119 (49.2%)	123 (50.8%)	0.91†
Yes	37 (30.3%)	85 (69.7%)		59 (48.4%)	63 (51.6%)	
Total	95 (25.8%)	273 (74.2%)		178 (48.9%)	186 (51.1%)	
Missing	42 (10.2%)			46 (11.2%)		

PSA=prostate specific antigen, GG=grade group, pStage=pathological stage, SVI=Seminal Vesicle Invasion, EPE=Extra-prostatic Extension, SM=Surgical Margin; * Log Rank test † Fisher exact test

Supplemental Table 3b. Association of PTEN and ERG immunohistochemistry status with clinicopathologic factors in the validation cohort

	PTEN status		p-value	ERG fusion		p-value
	Any loss	Intact		No	Yes	
Prostatectomy						
Grade Groups						
GG1	4 (6.8%)	55 (93.2%)	0.001	22 (40.0%)	33 (60.0%)	0.19
GG2	46 (27.7%)	120 (72.3%)		50 (30.7%)	113 (69.3%)	
≥GG3	4 (12.5%)	28 (87.5%)		7 (21.9%)	25 (78.1%)	
Total	54 (21.0%)	203 (79.0%)		79 (31.6%)	171 (68.4%)	
Missing	15 (5.8%)			22 (8.8%)		
Time to Recurrence						
No	32 (15.7%)	172 (84.3%)	< 0.001*	64 (32.3%)	134 (67.7%)	0.55*
Yes	22 (41.5%)	31 (58.5%)		15 (28.8%)	37 (71.2%)	
Total	54 (21.0%)	203 (79.0%)		79 (31.6%)	171 (68.4%)	
Missing	15 (5.8%)				22 (8.8%)	
pStage						
T2	31 (15.6%)	168 (84.4%)	0.001	68 (35.4%)	124 (64.6%)	0.06
T3a	20 (40.0%)	30 (60.0%)		9 (18.0%)	41 (82.0%)	
T3b	3 (37.5%)	5 (62.5%)		2 (25.0%)	6 (75.0%)	
Total	54 (21.0%)	203 (79.0%)		79 (31.6%)	171 (68.4%)	
Missing	15 (5.8%)			22 (8.8%)		
PTEN status						
No				12 (22.6%)	41 (77.4%)	0.14†
Yes				66 (33.7%)	130 (66.3%)	
Total				78 (31.3%)	171 (68.7%)	
Missing				23 (9.2%)		
SVI						
No	51 (20.5%)	198 (79.5%)	0.37†	77 (31.8%)	165 (68.2%)	1.00†
Yes	3 (37.5%)	5 (62.5%)		2 (25%)	6 (75%)	
Total	54 (21%)	203 (79%)		79 (31.6%)	171 (68.4%)	
Missing	15 (5.5%)				22 (8.8%)	
EPE						
No	31 (15.6%)	168 (84.4%)	<0.001†	68 (35.4%)	124 (64.6%)	0.02†
Yes	23 (39.7%)	35 (60.3%)		11 (19%)	47(81%)	
Total	54 (21%)	203 (79%)		79 (31.6%)	121 (68.4%)	
Missing	15 (5.5%)				22 (8.8%)	
SM						
No	44 (20%)	176 (80%)	0.38†	70 (32.9%)	143 (67.1%)	0.34†
Yes	10 (27%)	27 (73%)		9 (24.3%)	28 (75.7%)	
Total	54 (21%)	203 (79%)		79 (31.6%)	121 (68.4%)	

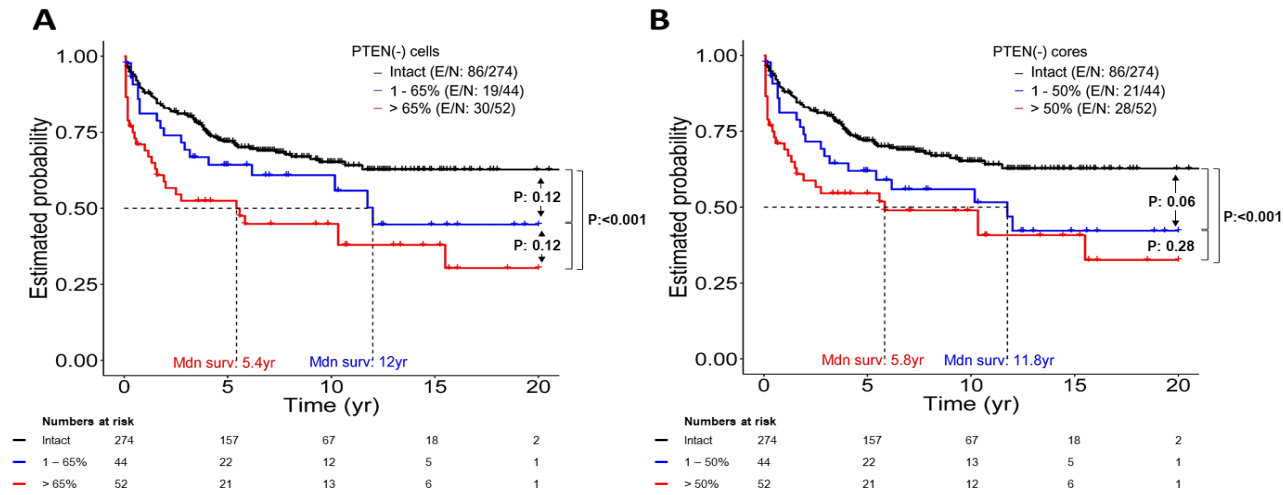
PSA=prostate specific antigen, GG=grade group, pStage=pathological stage, SVI=Seminal Vesicle Invasion, EPE=Extra-prostatic Extension, SM=Surgical Margin; * Log Rank test † Fisher exact test



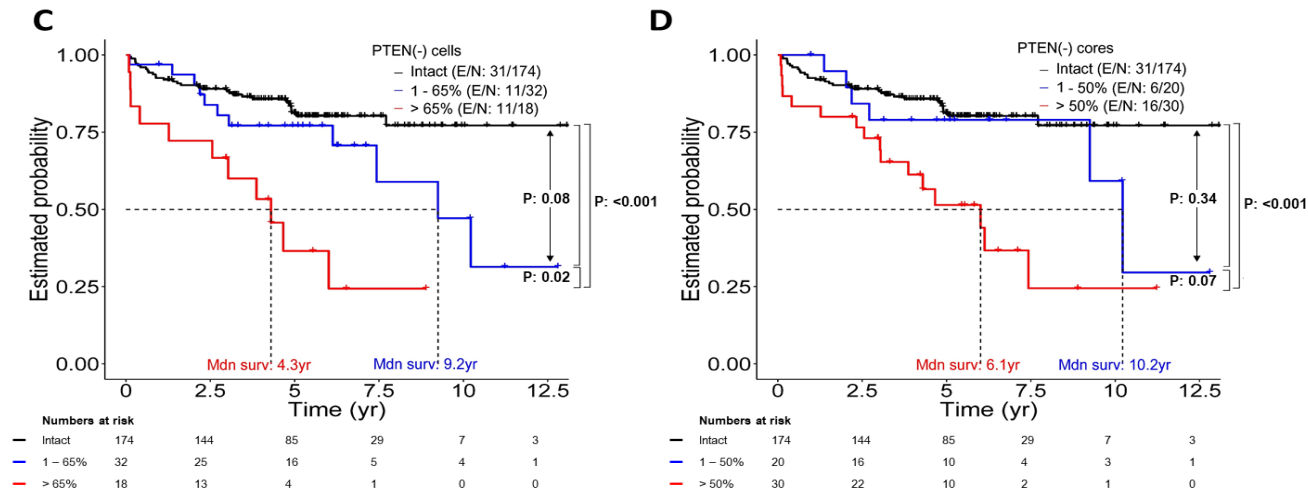
Supplemental Figure 1. Immunohistochemical staining patterns of PTEN and ERG. A, Intact PTEN; Tumor tissue has similar immunostaining intensity to surrounding stromal cells. B, Complete PTEN protein loss; PTEN protein is lost in 100% of sampled tumor cores. C, Partial PTEN protein loss; PTEN protein loss is seen in the subset of tumor cells (arrows). D, ERG positive core; ERG oncoprotein is markedly over-expressed. Staining intensity +3, % of positive cells 100% (homogeneous). Non-specific positive cytoplasmic staining/blush was ignored from scoring. E, ERG positive core; ERG protein is expressed but with less staining intensity (+2) compared to core A (number of positive cells 100% =homogeneous). Note, positive endothelial staining was used as internal control

(arrows). F, ERG negative core; ERG protein expression is completely negative (staining intensity 0). Tumor regions are indicated as “T” on the images, and benign regions as “B”. Scale bars=150.0um

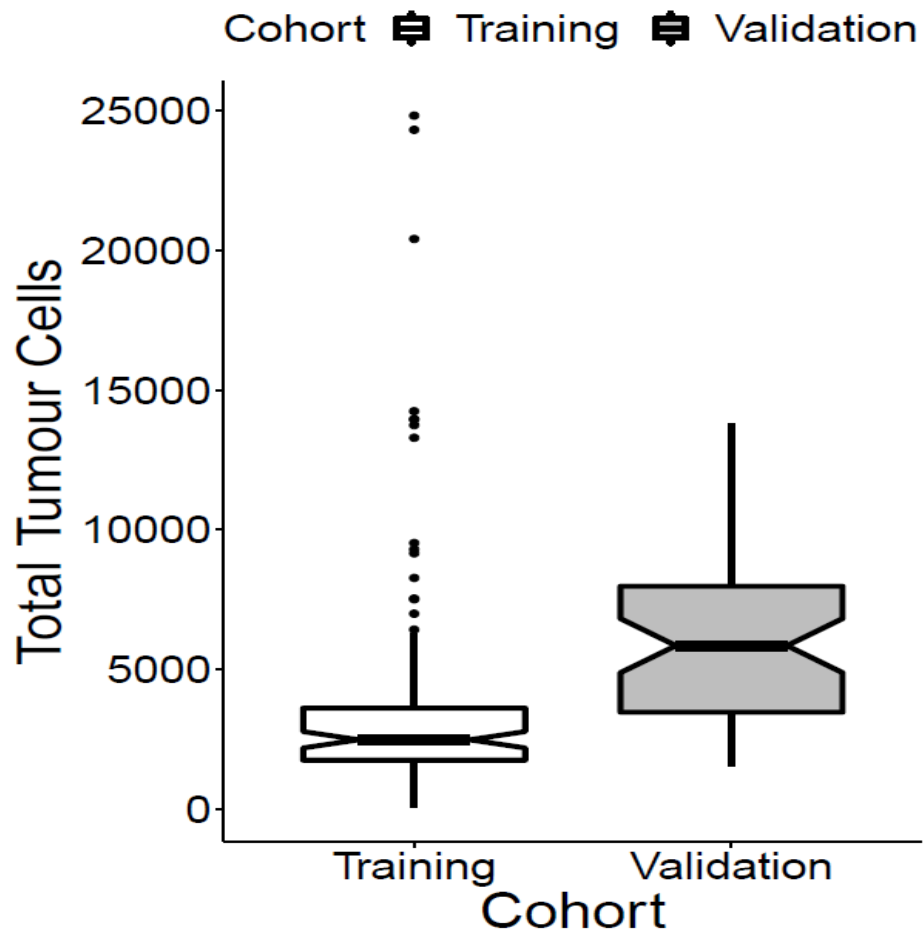
Training Cohort



Validation Cohort



Supplemental Figure 2. Kaplan-Meier curves for biochemical recurrence-free survival stratified by qPTEN thresholds. Training cohort data is represented in panels A-B and validation cohort data is shown in panels C-D.



Supplemental Figure 3: Boxplot showing distributions of data=tumor cells in sampled tissue cores from training and validation cohorts.