Supporting Information

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Detection of CTC Clusters and a Dedifferentiated RNA-Expression Survival Signature in Prostate Cancer

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Materials and Methods

Cell culture: The prostate cancer cell line PC-3 cell line was used to generate a scanning electron microscopy image to demonstrate cell capture on the GO Chip. This cell line was a generous gift from the lab of Mark L. Day following their purchase from ATCC. They were maintained in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (FBS, Corning) and 1% antibiotic-antimycotic (Gibco). Cells were maintained in a 37°C incubator with 5% CO_2. The cell line tested negative for Mycoplasma contamination as assayed using the Lonza Mycoalert on February 27, 2017.

Scanning electron microscopy: To fix cells on the device for scanning electron microscopy (SEM) imaging, the PDMS layer was removed and the substrate was fixed in 2.5% glutaraldehyde (Electron Microscopy Sciences) for one hour. The substrate was then washed twice with PBS and dehydrated through successive 10 minute dips in increasing concentrations of ethanol (50%, 70%, 95%, 100% twice). The substrate was dipped twice in hexamethyldisilazane (HDMS, Electron Microscopy Sciences) for 10 minutes each and then dried overnight in a chemical hood. The fixed substrate was then sputter coated with carbon and imaged on a FEI Nova 200 Nanolab.

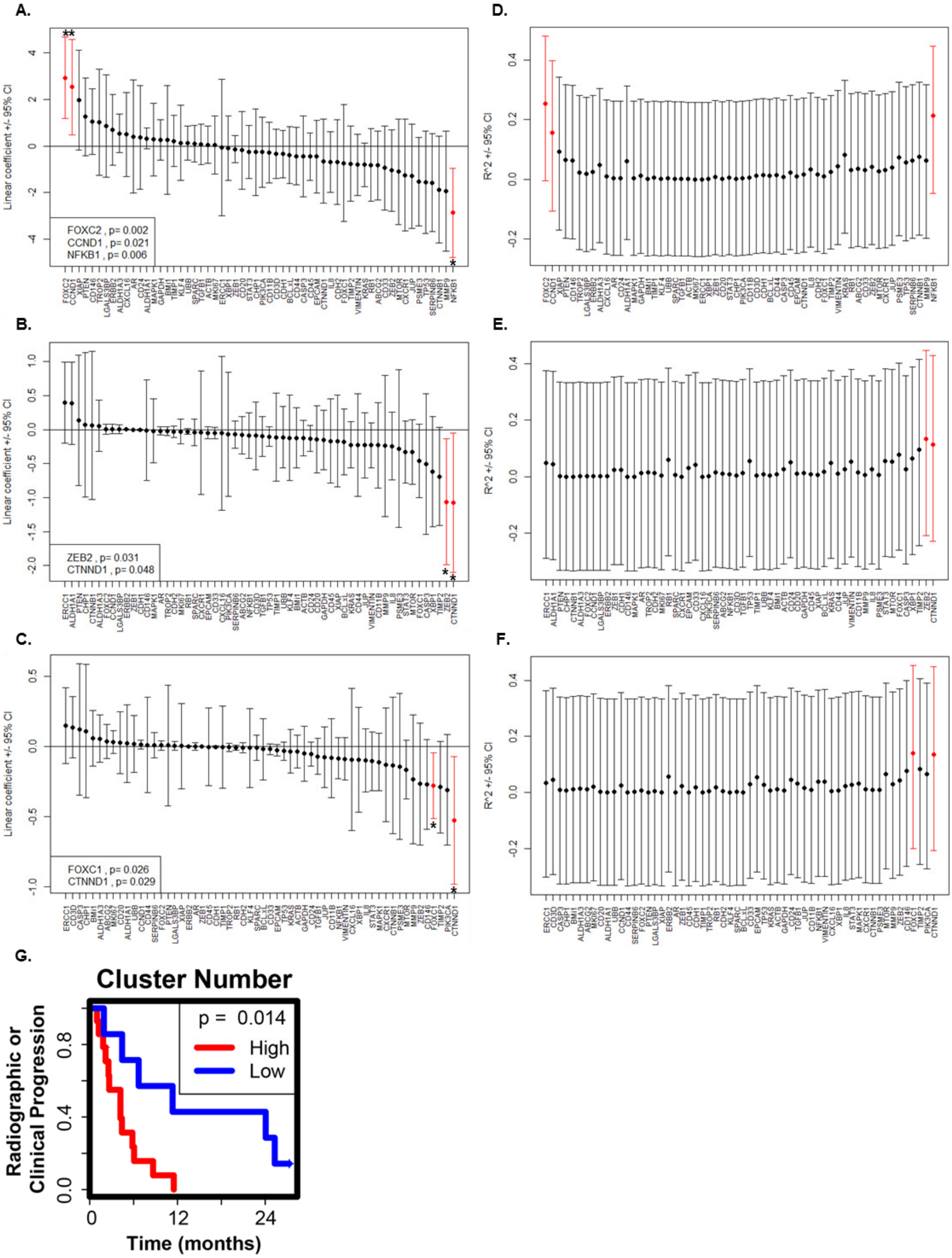


Figure S1. Modeling based on cluster metrics. A) Linear association of gene expression with number of CTCs/mL. Two genes, FOXC2 and CTNND1 showed a significant positive association while NKFB1 showed a significant negative association. B) Linear association of gene expression with number of CTC clusters. Two genes, CTNND1 and ZEB2, showed a significant negative association. C) Linear association of gene expression with percentage of

CTCs in clusters versus present as single cells. FOXC1 showed a significant positive association while CTNND1 showed a significant negative association. D, E, F) R^2 values for linear associations of gene expression with number of CTCs/mL, number of CTC clusters, and percentage of CTCs in cluster versus present as single cells, respectively. Data is plotted as the linear coefficient or R^2 ± the 95% confidence interval; */red denotes $p < 0.05$. G) Relationship between radioclinical progression and number of clusters as determined through Kaplan-Meier analysis ($p < 0.05$).

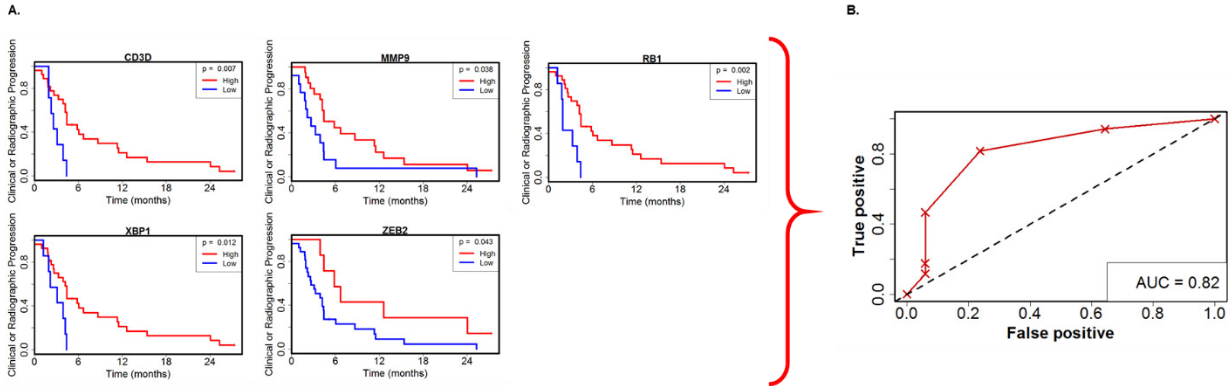


Figure S2. Relationship between RNA expression and radioclinical progression. A) Kaplan-Meier curves for all genes with statistically significant relationships with radiographic or clinical progression used to construct the optimized point-based score. B) Scores were optimized by maximizing the area under the curve (AUC) of the associated receiver operating curve.

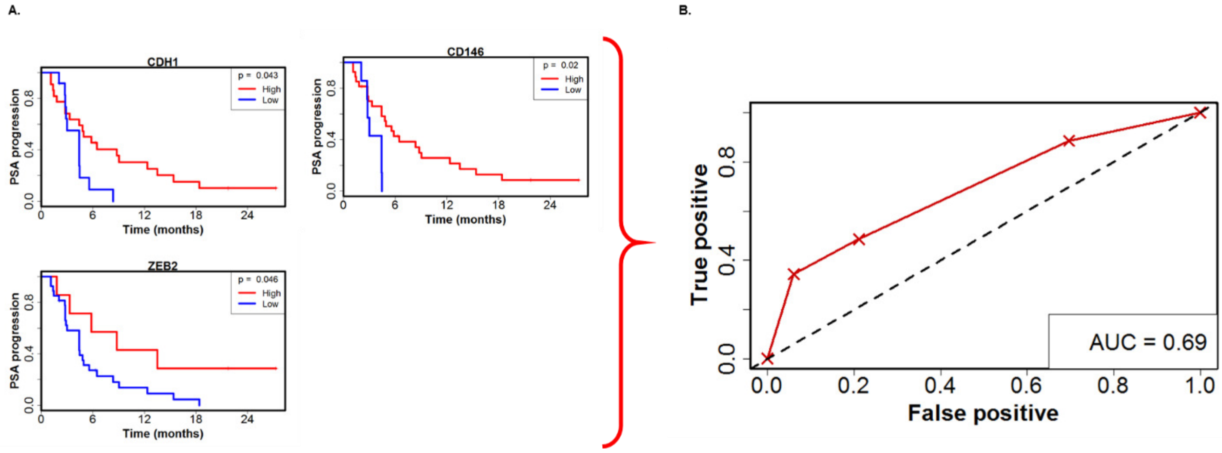


Figure S3. Relationship between RNA expression and PSA progression. A) Kaplan-Meier curves for genes with statistically significant relationships with overall survival used to construct the optimized point-based score. B) Scores were optimized by maximizing the area under the curve (AUC) of the associated receiver operating curve.

Table S1. Clinical cohort information.

Number of patients	41	100%
Age		
Median (range)	73	(50-84)
Race		
Caucasian	39	95.1%
Black	3	7.3%
Castrate resistant	41	100%
Performance status		
0	15	36.6%
1	22	53.7%
2	3	7.3%
Prostate-specific antigen (ng/mL)		
Median (range)	37.9	(1.2-6433.1)
Metastatic disease	41	100%
Visceral metastases	11	26.8%
Number of prior therapies		
Median (range)	1	0-7
Therapy at time of collection		
Abiraterone	17	41.5%
Cabazitaxel	4	9.8%
Cabozantinib	2	4.9%
Docetaxel	7	17.1%
Enzalutamide	8	19.5%
Olaparib	1	2.4%
Pembrolizumab	1	2.4%

Table S2. CTC and WBC counts for patients and healthy controls. A median of 20 CTCs/mL (range: 3-166 CTCs/mL) and 3 epithelial cells/mL (range: 0-14 epithelial cells/mL) were captured from patient and healthy samples, respectively. A median of 437 WBCs/mL (range: 56-54261 WBCs/mL) and 595.5 WBCs/mL (range: 359-2473 WBCs/mL) were non-specifically bound from patient and healthy samples, respectively.

Sample	CTCs/mL	WBCs/mL
1019	20	107
1021	53	264
1043	9	1292
1049	20	2169
1064	3	308
1074	33	482
1078	18	5938
1079	7	56
1081	10	355
1085	11	631
1087	25	690
1092	24	421
1093	18	418
1097	21	499
1103	8	243
1104	16	1034
1110	21	398
1128	25	878
1132	14	54261
1241	5	199
1246	7	344
1248	20	6050
1283	42	411
1285	14	509
1291	44	282
1299	4	199
1300	26	821
1319	29	8394
1328	28	6175
1330	8	341
1353	166	295
1357	24	289
1362	7	437
1371	34	902
1381	21	412
1384	5	10948
1399	55	558
1401	31	259
1407	17	329
1409	29	707
1411	62	1058
HC-1	3	1234
HC-2	14	645
HC-3	5	546
HC-4	5	678
HC-5	1	2473
HC-6	3	392
HC-7	0	454
HC-8	1	359

Table S3. Sample flow through study

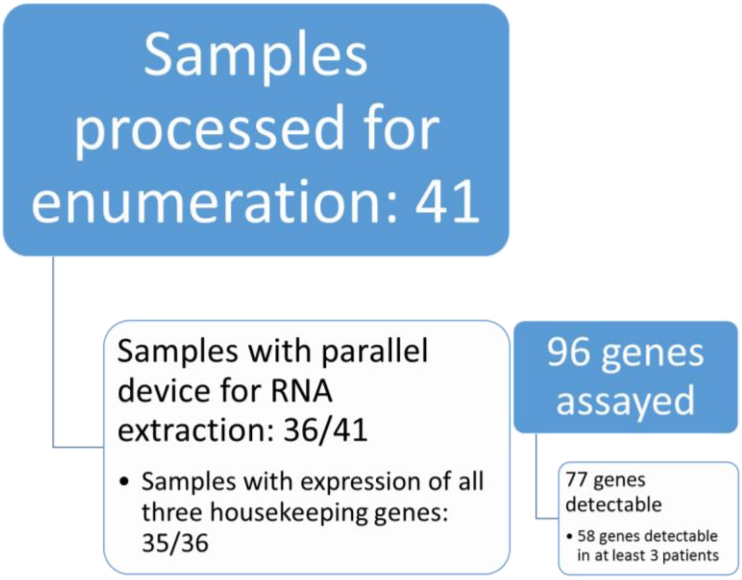


Table S4. List of 96 genes analyzed by RT-qPCR

Gene	Category
BCL-xL	Apoptosis
CASP3	Apoptosis
XIAP	Apoptosis
CD3D	Blood cell
CD11B	Blood cell
CD20	Blood cell
CD45	Blood cell
CD33	Blood cell
CD34	Blood cell
PTPRN2	Cell cycle
CCND1	Cell cycle
CHP1	Cell cycle
KLF4	Cell cycle
PSME3	Cell cycle
DSP	Cell junction
EVPL	Cell junction
JUP	Cell junction
CTNNA1	Cytoskeleton
CTNNB1	Cytoskeleton
CTNND1	Cytoskeleton
PKP2	Cytoskeleton
FOXC1	Transcription factor
FOXC2	Transcription factor
ERCC1	DNA repair
COL1A2	ECM
COL3A1	ECM
LGALS3BP	ECM
MMP2	ECM
MMP9	ECM
SPARC	ECM
TIMP1	ECM
TIMP2	ECM
CDH1	Epithelial
EPCAM	Epithelial
KRT7	Epithelial
KRT8	Epithelial
KRT14	Epithelial
KLK3	Epithelial
EMP2	Epithelial
MUC1	Epithelial
TROP2	Epithelial
FGF18	Growth factor
IGFBP5	Growth factor
TGFB1	Growth factor
AR	Hormone
GAPDH	Housekeeping
ACTB	Housekeeping
UBB	Housekeeping
CXCL16	Inflammation/Immune system
CXCR1	Inflammation/Immune system
IL6	Inflammation/Immune system
IL8	Inflammation/Immune system
MTOR	Inflammation/Immune system
NFKB1	Inflammation/Immune system
PD-1	Inflammation/Immune system
PDL-1	Inflammation/Immune system
XBP1	Inflammation/Immune system
XIST	lncRNA

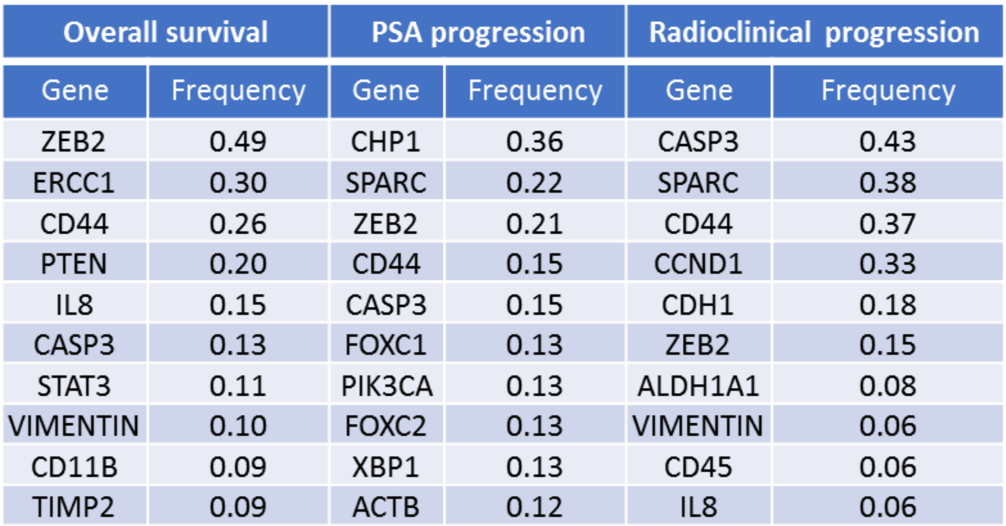
HOTAIR	lncRNA
CDH2	Mesenchymal
CDH11	Mesenchymal
VIMENTIN	Mesenchymal
KRT5	Mesenchymal
SNAI1	Mesenchymal
SNAI2	Mesenchymal
ZEB1	Mesenchymal
ZEB2	Mesenchymal
CD146	Mesenchymal/endothelial
ERBB2	Oncogene
ERG	Oncogene
FOLH1	Oncogene
ALK	Oncogene
EGFR	Oncogene
KRAS	Oncogene
MAPK1	Oncogene
NTRK2	Oncogene
PIK3CA	Oncogene
MKI67	Proliferation
MLPH	Signal transduction
SERPINB6	Signal transduction
STAT3	Signal transduction
ALDH1A1	Stemness
ALDH1A3	Stemness
CD24	Stemness
CD44	Stemness
CD44v6	Stemness
CD133	Stemness
BMi1	Stemness
ABCG2	Stemness/Drug resistance
ETV1	Transcription factor
TMPRSS2	Transcription factor
ELF3	Transcription factor
TP53	Tumor suppressor
PTEN	Tumor suppressor
RB1	Tumor suppressor
PTCH1	Tumor suppressor

Table S5. Detected genes

Genes detected in one or more patients ($C_T < 30$)	Detected in three or more patients?
ABCG2	Yes
ACTB	Yes
ALDH1A1	Yes
ALDH1A3	Yes
AR	Yes
BCL-xL	Yes
BMi1	Yes
CASP3	Yes
CCND1	Yes
CD11B	Yes
CD146	Yes
CD20	Yes
CD24	Yes
CD33	Yes
CD3D	Yes
CD44	Yes
CD45	Yes
CDH1	Yes
CDH2	Yes
CHP1	Yes
CTNNB1	Yes
CTNND1	Yes
CXCL16	Yes
CXCR1	Yes
EPCAM	Yes
ERBB2	Yes
ERCC1	Yes
FOXC1	Yes
FOXC2	Yes
GAPDH	Yes
IL8	Yes
JUP	Yes
KLF4	Yes
KRAS	Yes
LGALS3BP	Yes
MAPK1	Yes
MKI67	Yes
MMP9	Yes
MTOR	Yes
NFKB1	Yes
PIK3CA	Yes
PSME3	Yes
PTEN	Yes
RB1	Yes
SERPINB6	Yes
SPARC	Yes
STAT3	Yes
TGFB1	Yes
TIMP1	Yes
TIMP2	Yes
TP53	Yes
TROP2	Yes
UBB	Yes
VIMENTIN	Yes
XBP1	Yes
XIAP	Yes
ZEB1	Yes
ZEB2	Yes

CD44v6	No
DSP	No
EGFR	No
ELF3	No
EMP2	No
ETV1	No
EVPL	No
FOLH1	No
IGFBP5	No
IL6	No
KLK3	No
KRT8	No
MLPH	No
MUC1	No
PDL-1	No
PKP2	No
SNAI1	No
TMPRSS2	No
XIST	No

Table S6. Top ten genes appearing in multivariate Cox proportional hazards models. Frequency is reported as fraction of models for respective survival/progression metrics with global Wald p value of less than 0.05.



Overall survival		PSA progression		Radioclinical progression	
Gene	Frequency	Gene	Frequency	Gene	Frequency
ZEB2	0.49	CHP1	0.36	CASP3	0.43
ERCC1	0.30	SPARC	0.22	SPARC	0.38
CD44	0.26	ZEB2	0.21	CD44	0.37
PTEN	0.20	CD44	0.15	CCND1	0.33
IL8	0.15	CASP3	0.15	CDH1	0.18
CASP3	0.13	FOXC1	0.13	ZEB2	0.15
STAT3	0.11	PIK3CA	0.13	ALDH1A1	0.08
VIMENTIN	0.10	FOXC2	0.13	VIMENTIN	0.06
CD11B	0.09	XBP1	0.13	CD45	0.06
TIMP2	0.09	ACTB	0.12	IL8	0.06

Table S7. Breakdown of patients in high and low gene expression groups. Highlighted genes indicate those genes incorporated into gene scores.

	Gene	High expression	Low expression	Median PSA (ng/mL, High expression)	Median PSA (ng/mL, Low expression)	Median survival time (months, High expression)	Median survival time (months, Low expression)	Median no. prior therapies (High expression)	Median no. prior therapies (Low expression)
Overall survival	CD44	28	7	34.3	40.6	21.3	34.3	1	0
	CDH1	9	26	40.6	30.2	34.3	18.4	1	0.5
	EPCAM	8	27	32.9	37.5	27.3	21.3	0.5	1
	ERCC1	13	22	14.6	50.1	12.8	33.7	1	0.5
	IL8	26	9	35.2	37.45	21.3	34.3	0.5	1
	PIK3CA	13	22	14.6	40.6	17.7	27.3	1	0
	STAT3	7	28	30.2	37.5	N/A	21.3	0	1
	TGFB1	26	9	33.6	171.3	18.4	27.3	1	0
	TIMP2	25	10	18.95	97.85	18.4	34.3	0	1
	ZEB2	7	28	30.2	38.3	N/A	21.3	0	1

	Gene	High expression	Low expression	Median PSA (ng/mL, High expression)	Median PSA (ng/mL, Low expression)	Median progression free survival time (months, High expression)	Median progression free survival time (months, Low expression)	Median no. prior therapies (High expression)	Median no. prior therapies (Low expression)
PSA progression	CDH1	22	13	35.9	27.65	5.38	4.43	0	1
	CD146	28	7	40.6	11.7	5.6	3	0	1
	FOXC2	7	28	54.6	34.3	18.4	4.43	1	0.5
	ZEB2	7	28	30.2	38.3	8.8	4.43	0	1

	Gene	High expression	Low expression	Median PSA (ng/mL, High expression)	Median PSA (ng/mL, Low expression)	Median progression free survival time (months, High expression)	Median progression free survival time (months, Low expression)	Median no. prior therapies (High expression)	Median no. prior therapies (Low expression)
Radioclinical progression	ACTB	20	15	37.9	14.35	4.43	2.92	0.5	1
	CDH1	17	18	37.5	17	6.7	3.27	0	1
	CDH2	19	16	38.3	17	5.83	3.27	0	1
	CD3D	28	7	37.5	14.1	4.43	2.57	1	0
	CD45	25	10	35.9	28.8	4.43	2.25	0	1
	CASP3	27	8	37.5	17	4.43	3.27	1	0.5
	CD146	27	8	45.35	9.25	4.43	2.72	0	1
	CXCR1	28	7	38.3	11.7	4.43	3.27	0	1
	KLF4	22	13	37.9	14.35	5.13	2.3	0	1
	KRAS	28	7	37.5	17	4.43	2.57	1	0
	MKI67	22	13	39.05	15.55	4.43	3.18	0.5	1
	MMP9	22	13	38.3	17	5.83	2.67	0	1
	RB1	28	7	37.5	17	4.43	1.93	0	1
	SPARC	15	20	37.5	34.3	5.83	3.27	0	1
	XBP1	28	7	34.3	38.3	4.43	3.1	0	1
	ZEB2	7	28	30.2	38.3	6.7	3.93	0	1

Table S8. Antibody information.

Antibody	**Conjugate**	**Application**	**Company**	**Catalog number**	**Clone**
Goat anti-human EpCAM	Biotin	Cell capture	R&D Systems	BAF960	Polyclonal
Mouse IgG2a anti-human cytokeratin 7/8	N/A	Primary antibody	BD Biosciences	349205	CAM5.2
Mouse IgG1 anti-human CD45	N/A	Primary antibody	BD Biosciences	555480	HI30
Goat anti-mouse IgG2a	Alexa Fluor 546	Secondary antibody	ThermoFisher	A-21133	Polyclonal
Goat anti-mouse IgG1	Alexa Fluor 488	Secondary antibody	ThermoFisher	A-21121	Polyclonal