

Supplementary Information

Blockade of ACK1/TNK2 To Squelch the Survival of Prostate Cancer Stem-like Cells

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. RNAi screen of the tyrosine kinome to identify kinases essential for CD44⁺PSA^{-/lo} PCSCs survival.

(a) CD44⁺ LNCaP cells were isolated by sorting cells following staining with the CD44-PE antibodies, followed by fluorescence activated cell sorting (FACS). **(b)** Relative levels of PSA was determined by qRT-PCR. **(c)** Flow chart work-flow: CD44⁺PSA^{-/lo} PCSCs isolated from LNCaP were transfected with an siRNA library consisting of 3 sets of siRNAs for each of the 85 distinct receptor and non-receptor tyrosine kinases. After 48 hours, cells were harvested in culture media, stained with CD44-PE monoclonal antibody and DAPI to distinguish live/dead population and live cells were analyzed by flow cytometry.

Supplementary Figure S2. Network analysis of the 5 non-receptor tyrosine kinases identified in the siRNA screen to affect PCSCs survival.

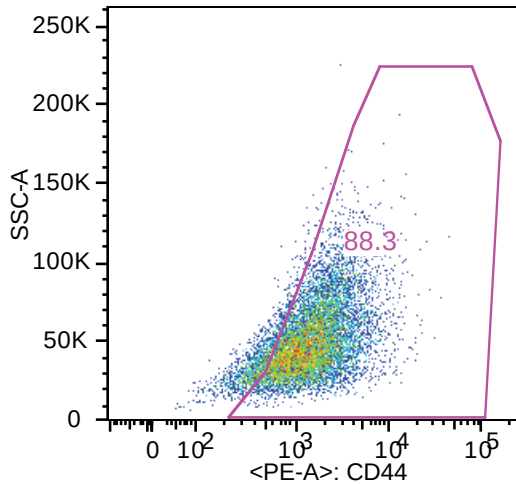
(a) 5 non-receptor tyrosine kinases identified in the siRNA (TNK2, TNK1, ALK, YES and TYK1) represent distinct nodal points in network analysis **(b)**. Bioinformatics analysis revealed a 3 kinase (ACK1/TNK2, TYK2 and YES1) Gene Set expression is altered in 39 of the 85 (45.9%) queried samples (TCGA). **(c)**. ACK1/TNK2 exhibited significant tendency towards co-occurrence with TYK2 ($p < 0.001$; Log Odds Ratio 2.113). In contrast, other kinases exhibited low tendency towards co-occurrence or tendency towards mutual exclusivity (c).

Supplementary Figure S3. ACK1 inhibitor (R)-9bMS inhibits radioresistant PCSC colony formation.

PCSCs were seeded in 6-well plate and irradiated at doses shown, followed by treatment with (R)-**9b**MS. After 10 days, the surviving cells were stained with crystal violet.

Supplementary Figure S1

a

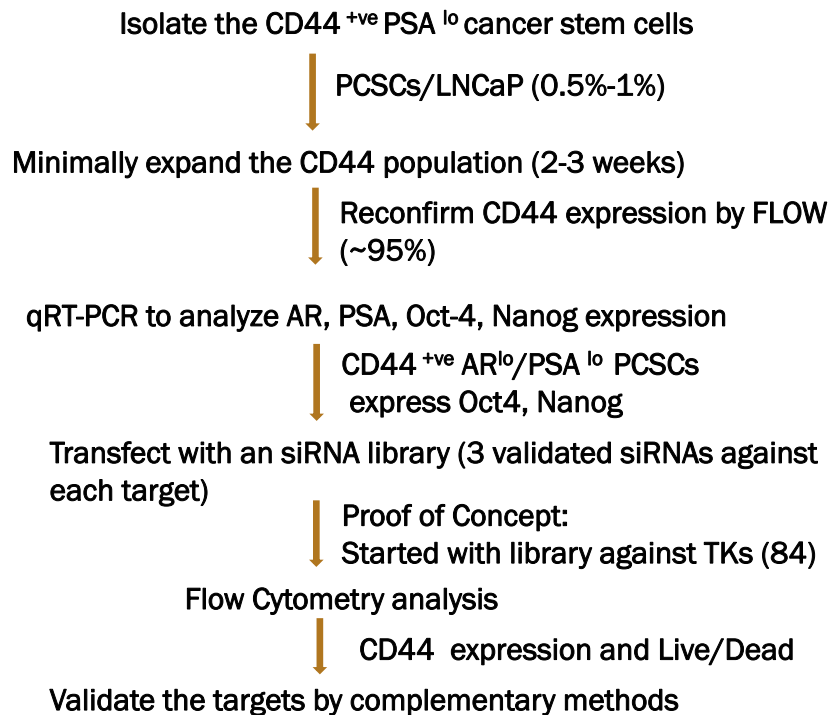


b

	Relative PSA levels
LNCaP	0.76
LNCaP CD44 ⁺	3.7x10 ⁻⁵

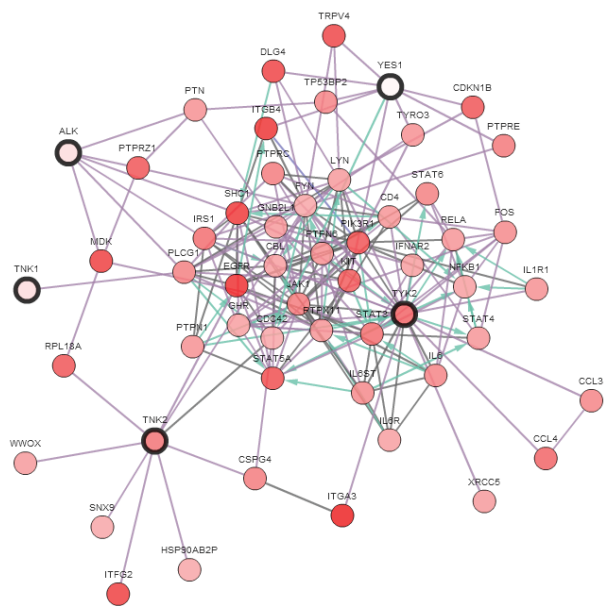
c

RNAi Screen of TK Kinome

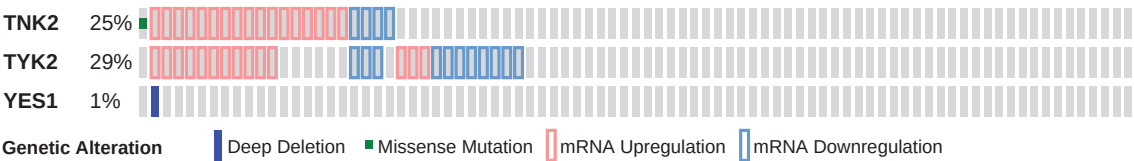


Supplementary Figure S2

a



b



c

Gene A	Gene B	p-Value	Log Odds Ratio	Association
TNK2	TYK2	<0.001	2.113	Tendency towards co-occurrence
TNK1	TNK2	0.107	1.572	Tendency towards co-occurrence
ALK	TYK2	0.167	<-3	Tendency towards mutual exclusivity
TNK2	ALK	0.214	<-3	Tendency towards mutual exclusivity
TNK2	YES1	0.259	>3	Tendency towards co-occurrence
TNK1	ALK	0.267	1.558	Tendency towards co-occurrence
YES1	TYK2	0.294	>3	Tendency towards co-occurrence
TNK1	TYK2	0.462	0.502	Tendency towards co-occurrence
TNK1	YES1	0.941	<-3	Tendency towards mutual exclusivity
YES1	ALK	0.941	<-3	Tendency towards mutual exclusivity

Supplementay Figure S3

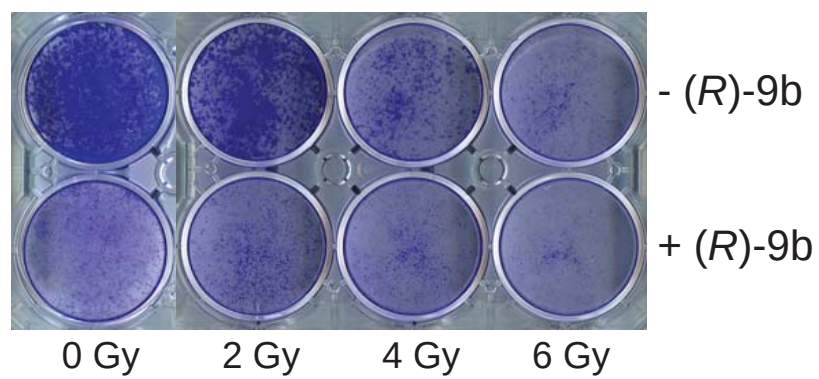


Table S1. Prostate TMA Data Analysis; CD44 expression by Disease Grade. Linear Association: no linear association between them observed (p-value by Mantel-Haenszel Chi-square test = 0.92).

CD44 level	Disease Grade					Total
	BPH	PIN	G6	G7/8/9/10	AI/Meta	
0	2 (8.3%)	2 (5.4%)	0 (0.0%)	15 (11.8%)	15 (55.6%)	34
2	4 (16.7%)	1 (2.7%)	0 (0.0%)	1 (0.8%)	0 (0.0%)	6
3	11 (45.8%)	1 (2.7%)	5 (15.2%)	10 (7.9%)	1 (3.7%)	28
4	7 (29.2%)	11 (29.7%)	3 (9.1%)	15 (11.8%)	4 (14.8%)	40
5	0 (0.0%)	13 (35.1%)	11 (33.3%)	28 (22.0%)	3 (11.1%)	55
6	0 (0.0%)	9 (24.3%)	13 (39.4%)	40 (31.5%)	2 (7.4%)	64
7	0 (0.0%)	0 (0.0%)	1 (3.0%)	13 (10.2%)	2 (7.4%)	16
8	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (3.9%)	0 (0.0%)	5
Total	24 (100%)	37 (100%)	33 (100%)	127 (100%)	27 (100%)	248

Pairwise Comparisons: At least one disease grade is different from others (p < 0.0001)

Disease Stage	N	Missing Freq.	Mean	SD	Median	Min	Max	p-value
BPH	24	0	2.88	1.12	3	0	4	<0.0001
PIN	37	3	4.54	1.45	5	0	6	
G6	33	1	5.06	1.12	5	3	7	
G7/8/9/10	127	7	4.75	2.12	5	0	8	
AI/Meta	27	4	2.22	2.67	0	0	7	

Adjusted p-values of pairwise comparisons by Tukey's Method and Interpretation

- BPH is significantly lower than PIN, G6, and G7/8/9/10.
- AI/Meta is significantly lower than PIN, G6, and G7/8/9/10.
- No difference between AI/Meta and BPH. No difference between PIN, G6, and G7/8/9/10.

	PIN	G6	G7/8/9/10	AI/Meta
BPH	0.009	0.0003	0.0002	0.74
PIN		0.79	0.98	<.0001
G6			0.92	<.0001
G7/8/9/10				<.0001

Table S2. Depletion of specific tyrosine kinases causes a decrease in live PCSC cells.

Kinase	% Live CD44 ⁺ Cells	Kinase	% Live CD44 ⁺ Cells	Kinase	% Live CD44 ⁺ Cells
ABL1	107	FES	102	MET	129
ABL2	113	FGFR1	110	MST1R	120
ALK	82	FGFR3	104	MSK	127
AXL	94	FGFR2	96	NTRK1	110
BLK	117	FGFR4	103	NTRK2	109
BMX	104	FGR	102	NTRK3	115
BTIC	115	FLT1	90	ROR1	132
DDR1	95	FLT3	104	ROR2	100
DDR2	109	FLT4	93	PDGFRA	91
PDGFRb	110	FRK	99	PTK2	94
CSK	105	FYN	101	PTK6	102
EGFR	107	HCK	98	PTK7	92
EPHA2	107	IGF1R	103	RET	95
EPHA1	116	INSR	105	ROS1	111
EPHA3	105	INSRR	104	RYK	121
EPHA4	115	ITK	103	SRC	104
EPHA5	111	JAK1	105	SRMS	132
EPHA7	117	JAK2	104	SYK	105
EPHA8	108	JAK3	103	TEC	105
EPHB1	108	KDR	105	TEK	119
EPHB2	101	KIT	97	TIE1	103
EPHB3	107	LMTK2	105	TNK1	73
EPHB4	110	LTK	101	ACK1	64
EPHB6	102	LYN	105	TXK	99
ERBB2	96	MATK	104	TYK2	84
ERBB3	89	Control	100	TYRO3	101
ERBB4	107			WEE1	92
PTK2B	92			YES1	85
FER	104			ZAP70	110
Control	100			Control	100