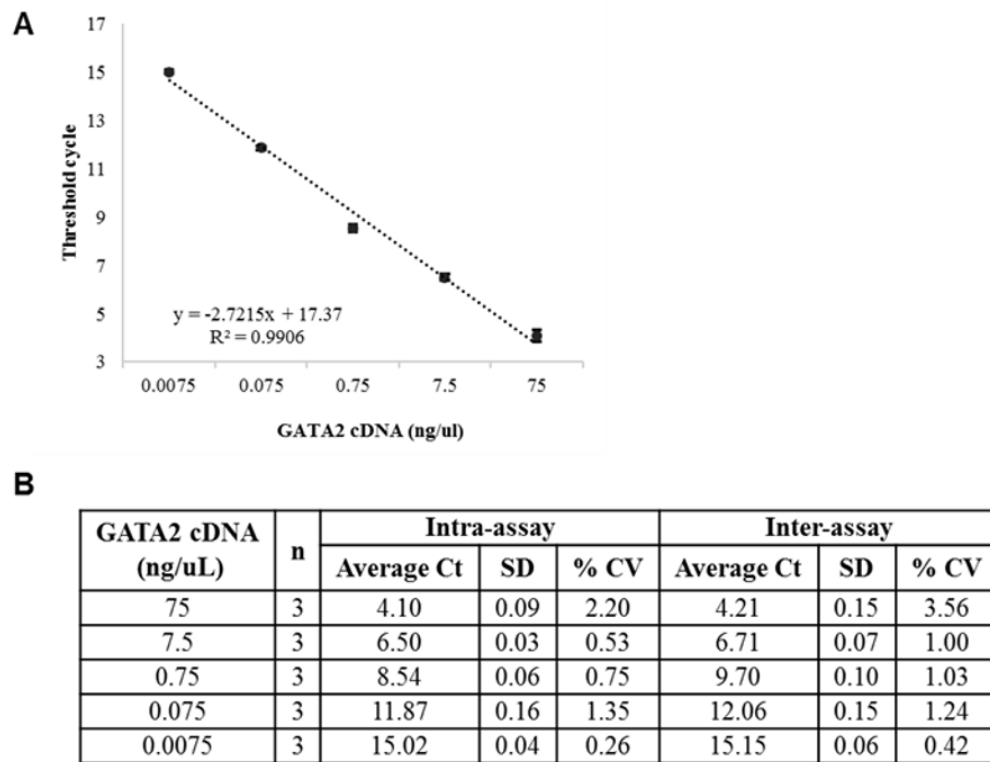
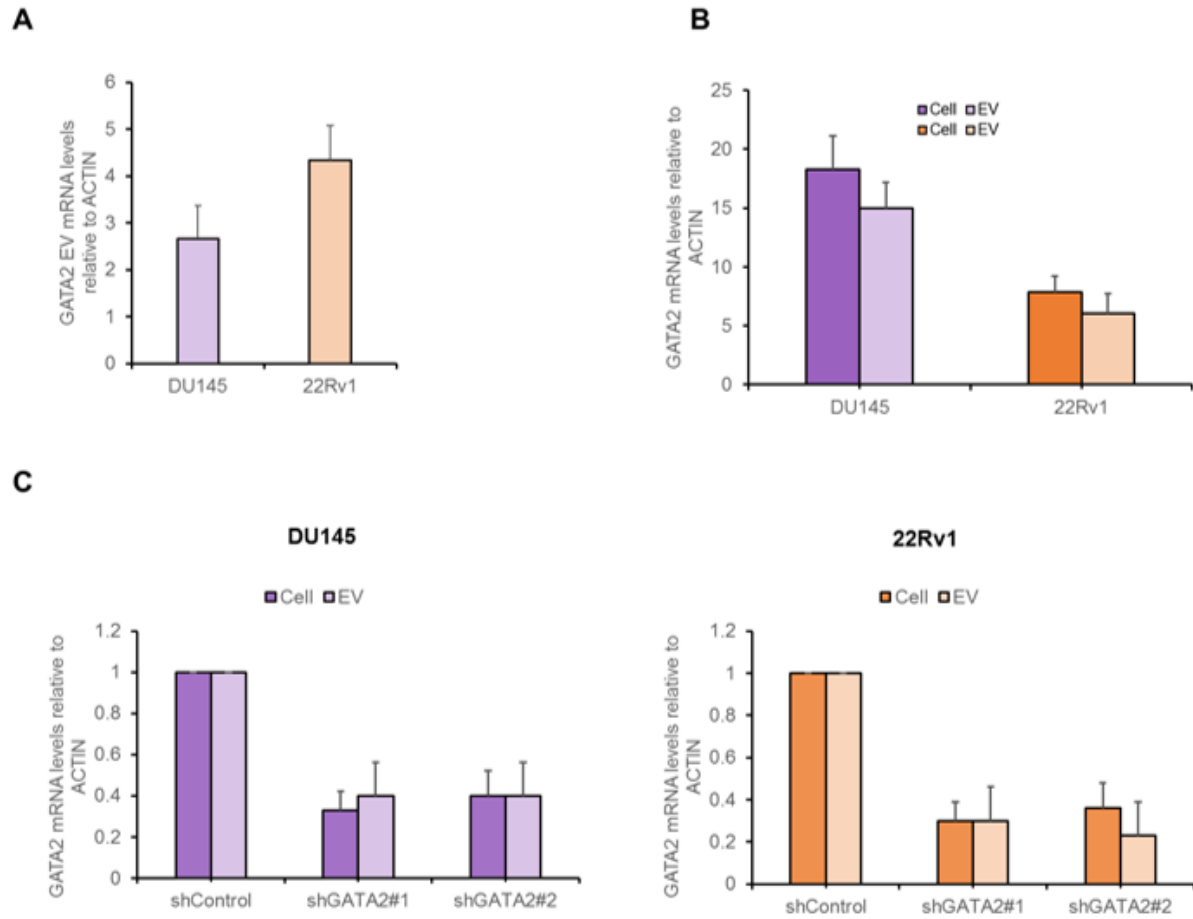


## Supplementary Data



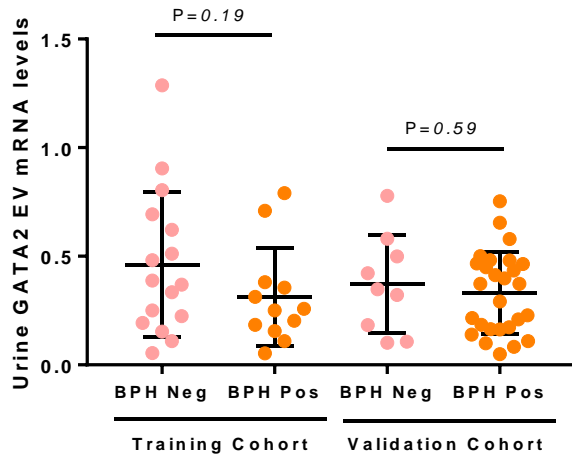
**Fig. S1. A)** Standard curve containing serial dilutions of GATA2 cDNA from 0.0075 to 75ng/ $\mu$ L. Serial dilutions were analyzed in triplicate. The y-axis plots Ct values and the x-axis plots cDNA input. **B)** GATA2 RT-PCR intra- and inter-assay reproducibility.

SD= standard deviation; CV= coefficient variation



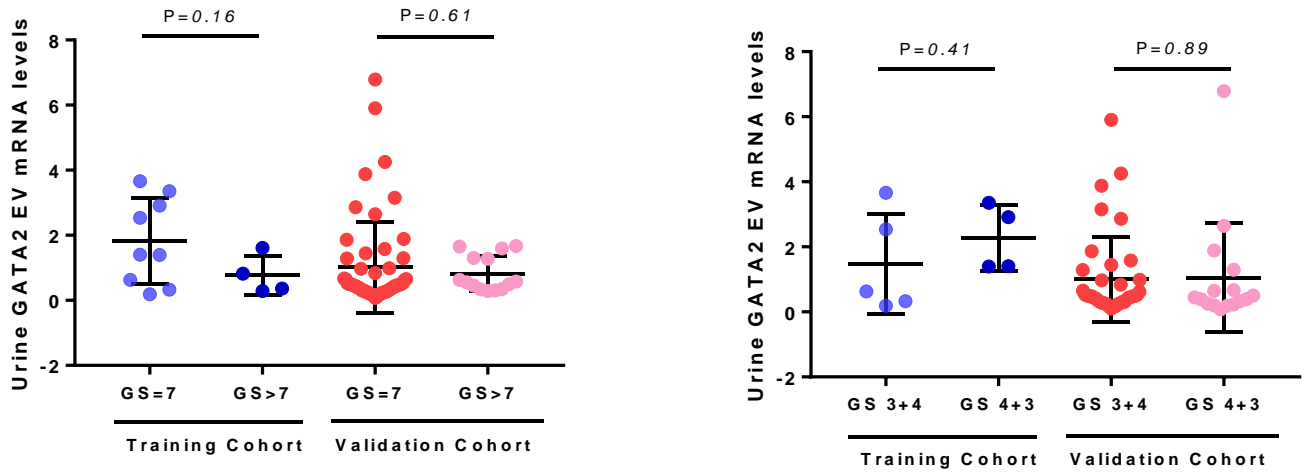
**Fig. S2. A)** EV GATA2 mRNA expression levels from the conditioned media of PCa cells (DU145 and 22Rv1). EV GATA2 mRNA expression levels in paired PCa cells and conditioned media in **B)** GATA2-overexpressing and **C)** GATA2-depleted PCa cells.

EV = extracellular vesicle



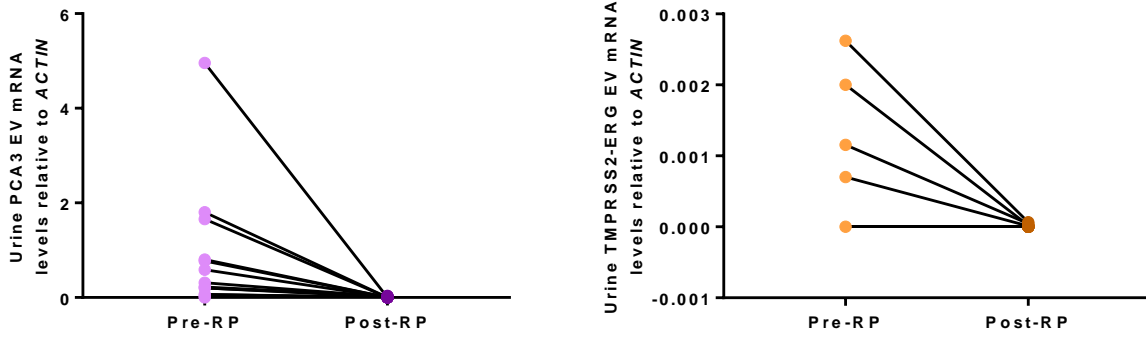
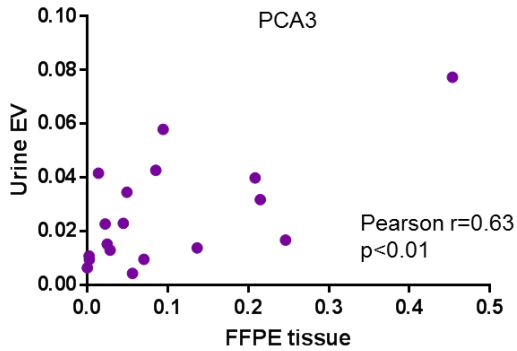
**Fig. S3.** Analysis of EV GATA2 mRNA in patients with negative biopsy result with or without Benign Prostatic Hyperplasia (BPH) (t-test).

EV = extracellular vesicle



**Fig. S4.** Analysis of urine EV GATA2 mRNA in **A)** Gleason 7 vs >7 and **B)** Gleason 3+4 vs 4+3 PCa (t-test) training and validation cohorts.

EV = extracellular vesicle

**A****B**

		TMPRSS2-ERG		Fisher test p-value <0.05
		Tissue -	Tissue +	
Urine -	9	1		
Urine +	2	7		

**Fig. S5.** Analysis of EV PCA3 and TMPRSS2-ERG mRNA in **A**) paired pre- and post-RP urine samples (n=16, t-test) and in **B**) matching urine and RP FFPE tissue (n=19, Pearson correlation for PCA3 and Fisher test for TMPRSS2-ERG).

EV = extracellular vesicle; RP = radical prostatectomy; FFPE = formalin-fixed paraffin-embedded

**Table S1. Primer sequences used for RT-qPCR.**

<b>Gene</b>	<b>Forward Sequence (5' to 3')</b>	<b>Reverse Sequence (5' to 3')</b>
<b>GATA2</b>	CCCTAAGCAGCGCAGCAAGGC	GATGAGTGGTCGGTTCTGCCC
<b>PCA3</b>	GCACATTTCCAGCCCCTTA	GGCATTCTCCCAGGGATCT
<b>TMPRSS2-ERG</b>	TAGGCGCGAGCTAAGCAGGAG	GTAGGCACACTCAAACAACGACTGG
<b>ACTIN</b>	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

**Table S2. Scoring Parameters for GAPT-E, PT-E, and SOC scores developed in the training cohort.**

	<b>Training</b>
<b>GAPT-E</b>	
Intercept	1.35
log(PCA3)	0.62
log(GATA2)	1.34
T-E (0 vs. >0)	-1.08
<b>PT-E</b>	
Intercept	1.17
log(PCA3)	1.27
T-E (0 vs. >0)	-0.70
<b>SOC</b>	
Intercept	-2.57
PSA (1 pt)	0.07
Family History (No vs Yes)	0.79
Age (1 year)	0.01

SOC=standard of care; T-E=TMPRSS2-ERG; PT-E= PCA3 plus TMPRSS2-ERG. GAPT-E= GATA2, PCA3, and TMPRSS2-ERG.

**Table S3. Performance (AUC, 95% CI) of urine EV biomarkers alone, SOC alone and combined.**

	<b>Training</b>	<b>Validation</b>
	<b>AUC (95% CI)</b>	<b>AUC (95% CI)</b>
<b>Cancer vs no cancer</b>		
GAPT-E	0.84 (0.72-0.96)	0.72 (0.63-0.81)
GAPT-E + SOC	0.88 (0.78-0.98)	0.72 (0.63-0.80)
PT-E	0.81 (0.66-0.94)	0.67 (0.58-0.77)
GATA2	0.74 (0.59-0.89)	0.66 (0.57-0.75)
PCA3	0.76 (0.61-0.90)	0.62 (0.53-0.72)
SOC	0.64 (0.48-0.81)	0.62 (0.52-0.72)
<b>GS 7 vs GS 6 or no cancer</b>		
GAPT-E	0.85 (0.71-0.99)	0.71 (0.63-0.80)
GAPT-E + SOC	0.90 (0.79-1.00)	0.75 (0.67-0.83)
PT-E	0.80 (0.65-0.96)	0.65 (0.56-0.74)
GATA2	0.78 (0.62-0.94)	0.66 (0.57-0.74)
PCA3	0.79 (0.64-0.94)	0.62 (0.52-0.71)
SOC	0.73 (0.55-0.90)	0.65 (0.55-0.74)

AUC=area under the curve; CI=confidence interval; SOC=standard of care; T-E=TMPRSS2-ERG; PT-E= PCA3 plus TMPRSS2-ERG. GAPT-E= GATA2, PCA3, and TMPRSS2-ERG.



**Table S4. Logistic regression model parameters used to generate ROC curves.**

	<b>Any Cancer</b>		<b>High-grade cancer</b>	
	<b>Training</b>	<b>Validation</b>	<b>Training</b>	<b>Validation</b>
<b>GAPT-E + SOC</b>				
Intercept	-2.00	2.00	-12.4	1.72
GAPT-E Score (1 point)	0.0592	0.0378	0.0612	0.0368
PSA (1 point)	0.08	-0.02	0.05	-0.02
Age (1 year)	-0.04	-0.02	0.02	-0.02
Family History (No vs Yes)	1.22	0.38	6.66	0.49
Race	-		-	
African American vs. Other	-	0.66	-	1.08
Caucasian vs. Other	-	-0.54	-	-1.18
<b>GAPT-E alone</b>				
Intercept	-2.69	0.03	-3.74	-0.74
GAPT-E Score (1 point)	0.0540	0.0372	0.0542	0.0349
<b>PT-E alone</b>				
Intercept	-2.66	-0.03	-3.59	-0.61
PT-E Score (1 point)	0.0528	0.0368	0.0533	0.0295
<b>SOC</b>				
Intercept	-2.57	3.23	-11.2	2.56
PSA (1 point)	0.07	-0.03	0.05	-0.04
Race	-	-	-	-
African American vs. Other	-	0.64	-	0.93
Caucasian vs. Other	-	-0.51		-1.08
Age (1 year)	0.01	-0.02	0.05	-0.02
Family History (No vs Yes)	0.79	0.31	6.34	0.34
<b>PCA3</b>				

Intercept	0.98	2.25	0.30	1.19
log(PCA3)	1.05	0.77	1.19	0.58
<b>GATA2</b>				
Intercept	0.49	1.79	-0.33	1.10
log(GATA 2)	1.13	0.73	1.20	0.81

SOC=standard of care; T-E=TMPRSS2-ERG; PT-E= PCA3 plus TMPRSS2-ERG. GAPT-E=  
GATA2, PCA3, and TMPRSS2-ERG.