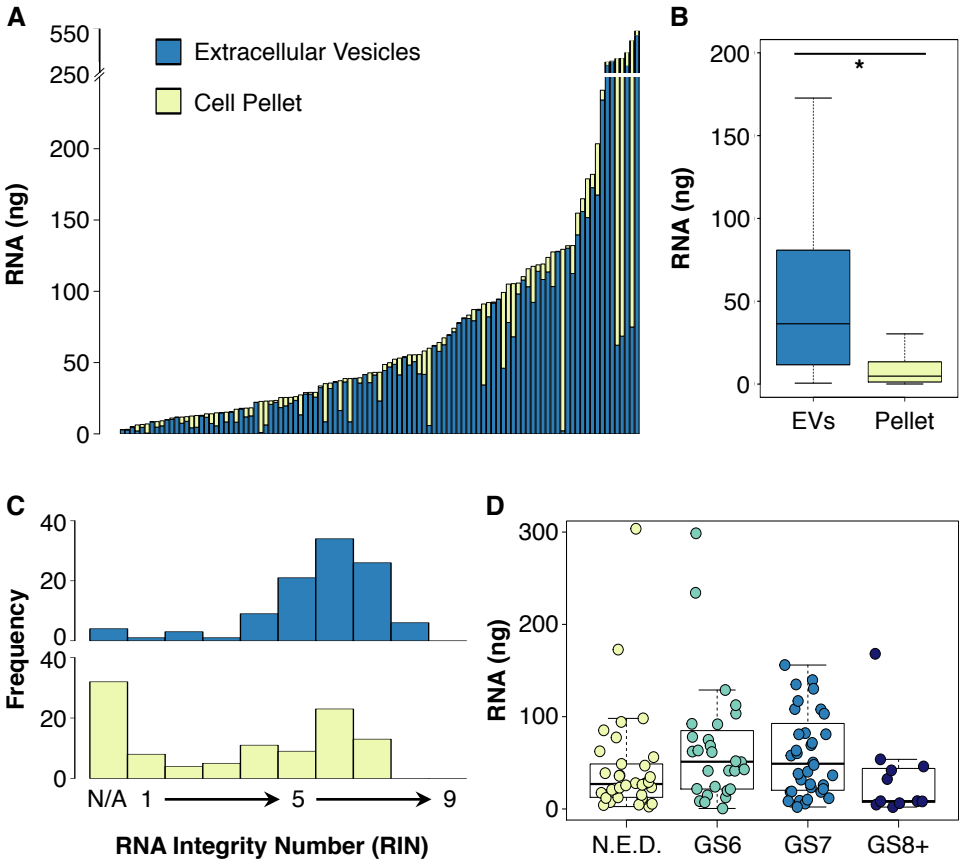


Figure 1. RNA yield from the pellet and extracellular vesicle (EV) fraction of post-DRE urine. (A) Urine was collected following a DRE and processed to isolate the cell pellet and EV fraction. For each sample, the amount of RNA extracted is represented as a stacked bar, with the amount isolated from the EVs shown in dark gray while the amount of RNA isolated from the cell pellet is shown in light gray (n = 105). The y-axis has been split to improve visualization of the low yield samples. (B) Summary data of the data in A is represented with boxplots (outliers are not shown). The RNA yield was significantly higher in the EVs ($p < 0.001$). (C) The frequency of RNA quality is shown for EVs (upper plot) and cell pellets (lower plot). The RIN scale goes from 1 to 10, with samples of particularly low quality or concentration unable to have RINs determined (shown as N/A). Representative Bioanalyzer plots are shown in Supplementary Figure 2. (D) The yield of EV RNA was not significantly different amongst patients with no evidence of disease (N.E.D.) or various levels of prostate cancer risk ($p = 0.275$; n = 34, 28, 36, and 11; one outlier in the GS7 group at 506 ng not shown).

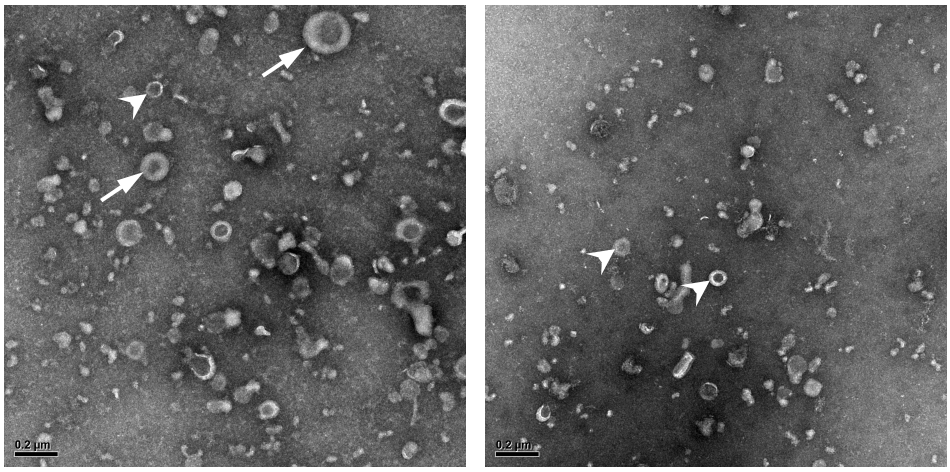


Figure 2. Analysis of urinary extracellular vesicles (EVs) by TEM. The EV fractions recovered from two different patients were negatively-stained and imaged by TEM. The observed particles include larger microvesicles (arrows) as well as smaller vesicles likely to be exosomes (arrowheads; scale bar on both images represents 200 nm).

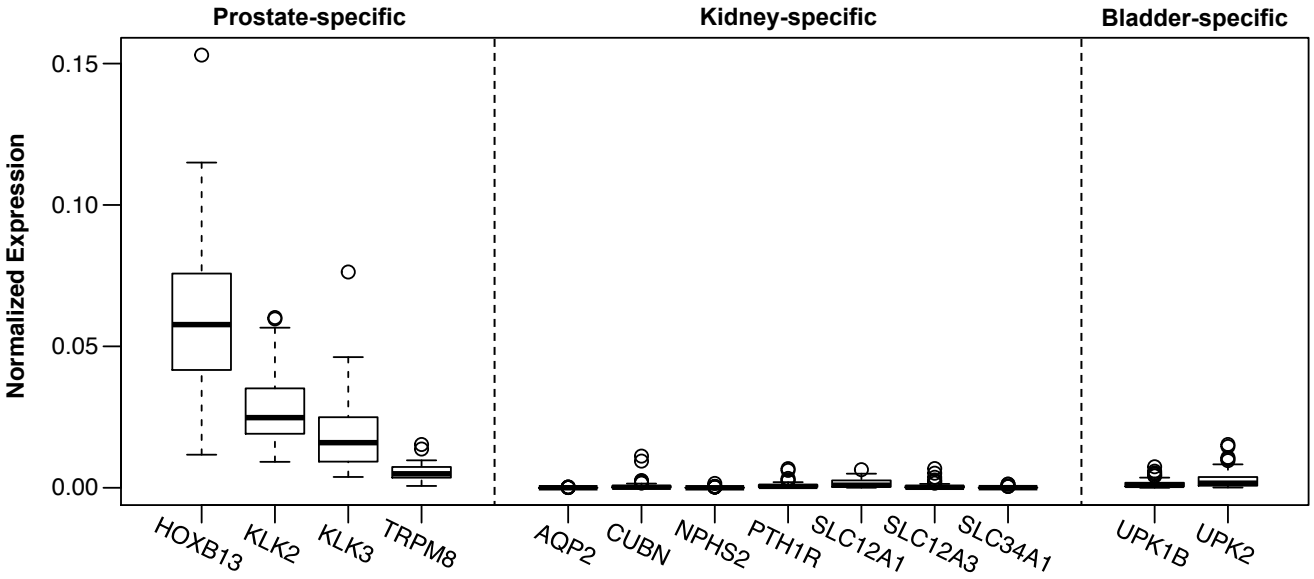


Figure 3. Prostate-specific genes are enriched in post-DRE urine EVs. The expression of various prostate-, kidney-, or bladder-specific transcripts were measured by TaqMan qPCR and are shown relative to the expression of *RAB7A* (n = 60). The boxplots for *AQP2*, *CUBN*, *NPHS2*, and *SLC34A1* include data for samples where no amplification was observed and the cycle threshold was set to 40 (8, 1, 20, and 2 samples, respectively).

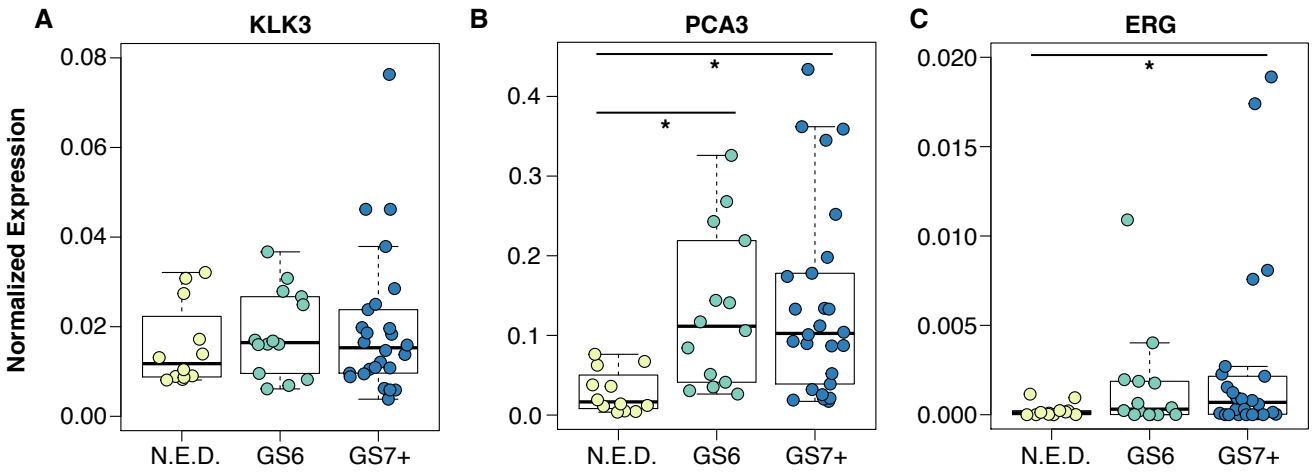
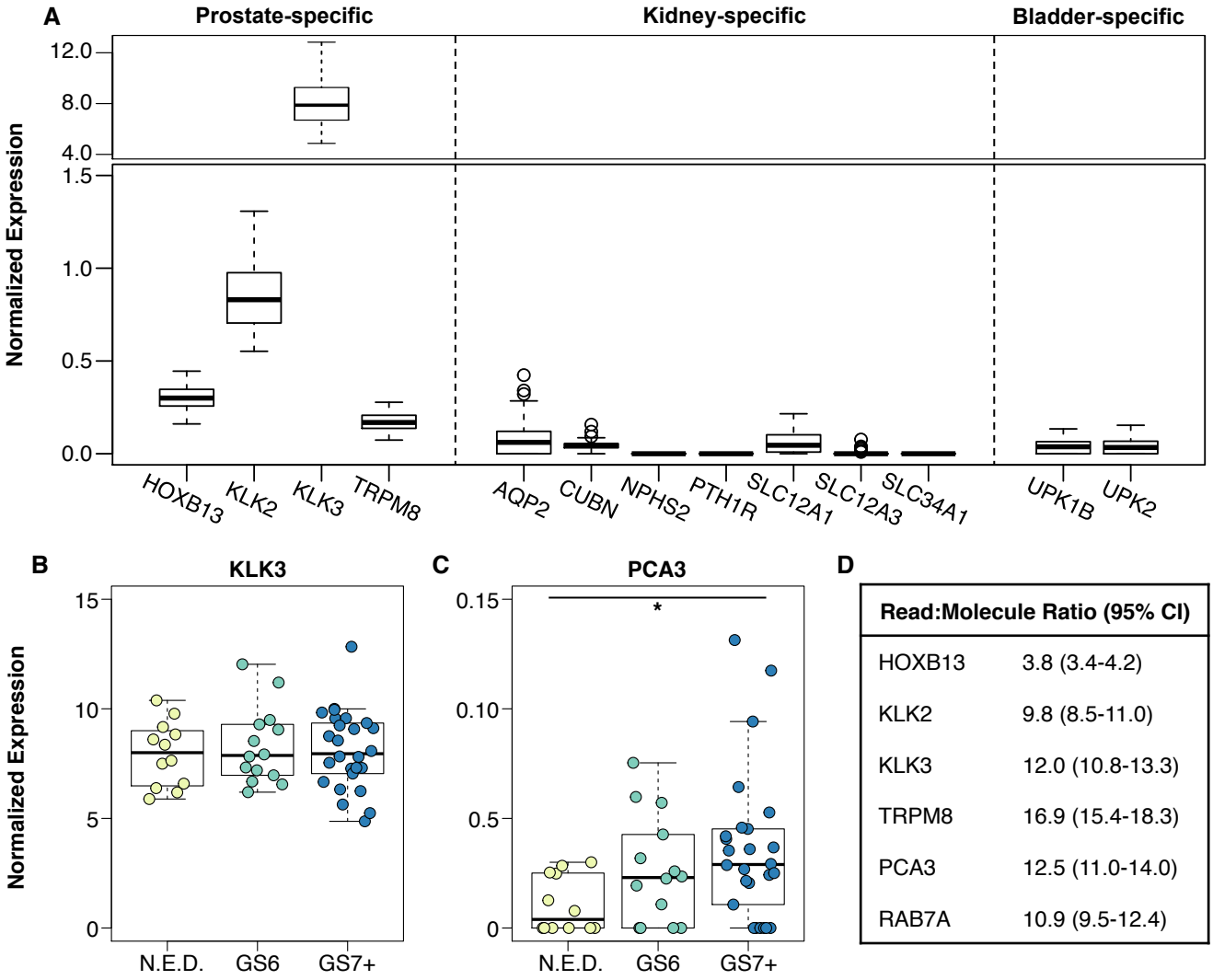


Figure 4. Prostate cancer-associated RNAs are detectable and informative in post-DRE urine EVs. The expression levels of (A) *KLK3*, (B) *PCA3*, and (C) *ERG* were measured by TaqMan qPCR assay and normalized to *RAB7A*. The data is shown for patients with no evidence of disease (N.E.D., n = 12), Gleason score 6 prostate cancer (GS6, n = 14), or Gleason score ≥7 prostate cancer (GS7+, n = 26). The *ERG* plot includes data for 9 samples where no amplification was observed and the cycle threshold was set to 40 (2, 2, and 5 samples for the N.E.D., GS6, and GS7+ groups, respectively). In comparison to the N.E.D. group, the GS6 and GS7+ groups both had significantly higher expression of *PCA3* ($p = 0.002$ and $p < 0.001$, respectively), while only the GS7+ group had significantly higher expression of ERG ($p = 0.027$). There was no significant difference in *KLK3* expression between the any of the groups ($p = 0.672$).



Read:Molecule Ratio (95% CI)	
HOXB13	3.8 (3.4-4.2)
KLK2	9.8 (8.5-11.0)
KLK3	12.0 (10.8-13.3)
TRPM8	16.9 (15.4-18.3)
PCA3	12.5 (11.0-14.0)
RAB7A	10.9 (9.5-12.4)

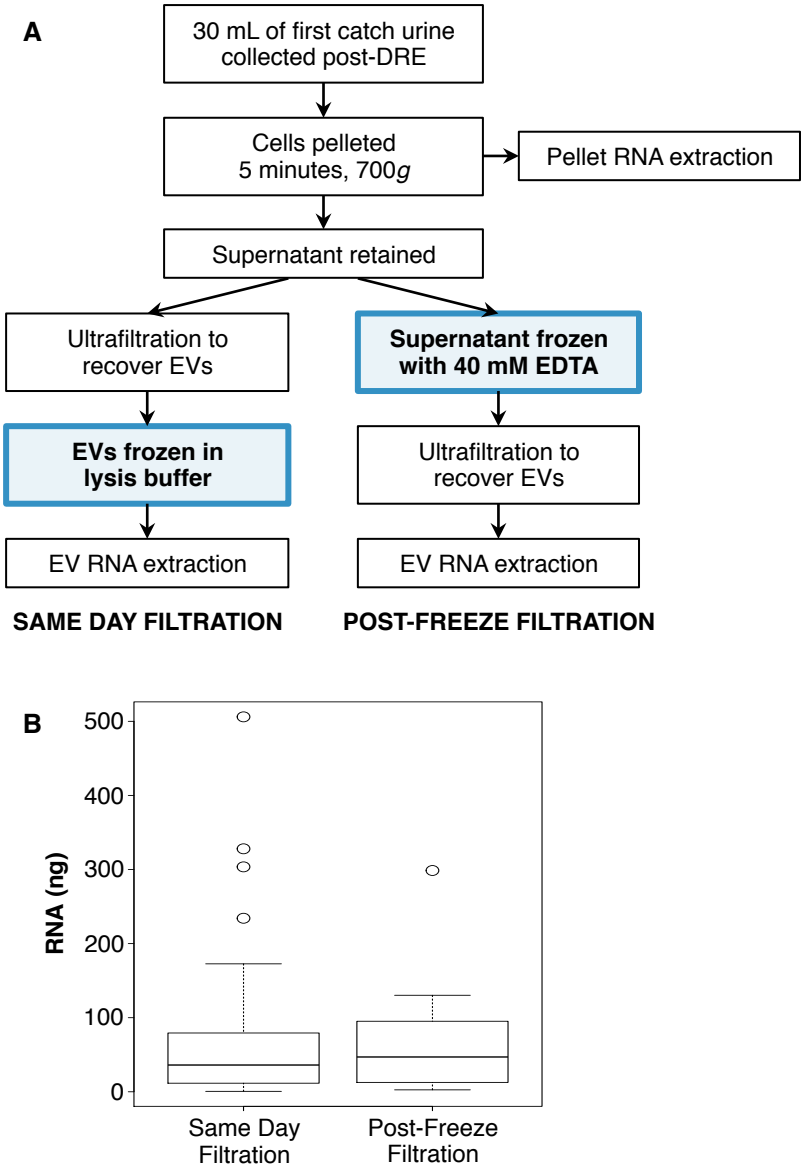
Figure 5. Precise Assay measurement of transcripts also shows enrichment of prostate-specific and prostate cancer-associated transcripts. (A) The same prostate-, kidney-, and bladder-specific transcripts shown in Figure 3 were assessed by Precise Assay and normalized to *RAB7A* (n = 60). *KLK3* is shown on a different scale because it was present at much higher levels than the other transcripts. The boxplots for *AQP2*, *CUBN*, *NPHS2*, *PTH1R*, *SLC12A1*, *SLC12A3*, *SLC34A1*, *UPK1B*, and *UPK2* include data for samples where no transcripts were detected and the normalized expression was set to 0 (17, 7, 60, 60, 15, 54, 60, 17, and 22 samples, respectively). Transcripts for (B) *KLK3* and (C) *PCA3* were measured by Precise Assay and normalized to *RAB7A* (n = 12, 14, and 26 for the N.E.D., GS6, and GS7+ groups, respectively). The *PCA3* plot includes data for 14 samples where no transcripts were detected and normalized expression was set to 0 (6, 4, and 6 samples for the N.E.D., GS6, and GS7+ groups, respectively). GS7+ patients had significantly higher expression of *PCA3* as compared to the N.E.D. group (p = 0.003). (D) The ratio of sequence reads to molecular index count is shown for all of the prostate-associated genes and the normalizer *RAB7A* (means and 95% confidence intervals shown).

Table 1. Patient characteristics

VARIABLE	LEVEL	COHORT					TOTAL
		N.E.D. N = 34	No Biopsy N = 11	Biopsy Negative N = 17	Biopsy Positive N = 46	RP N = 29	
Age (years)	Mean ± SD	51.9 ± 9	61.6 ± 8.6	66.0 ± 9.7	65.5 ± 8.9	60.5 ± 7.7	60.8 ± 10.3
Race	Asian	1 (2.9%)	-	1 (5.9%)	-	-	2 (1.5%)
	Black	16 (47.1%)	3 (27.3%)	5 (29.4%)	13 (28.3%)	9 (31.0%)	46 (33.6%)
	White	16 (47.1%)	8 (72.7%)	9 (52.9%)	29 (63.0%)	19 (65.5%)	81 (59.1%)
	Unknown	1 (2.9%)	-	2 (11.8%)	4 (8.7%)	1 (3.5%)	8 (5.8%)
PSA (ng/mL)	Mean ± SD	1.2 ± 0.9*	5.5 ± 2.9	11.1 ± 9.9	16.5 ± 53.9	7.2 ± 4.4	9.7 ± 32.8
Biopsy Gleason Score	6	-	-	-	24 (52.2%)	6 (20.7%)	30 (40.0%)
	7	-	-	-	18 (39.1%)	16 (55.2%)	34 (45.3%)
	8+	-	-	-	4 (8.7%)	7 (24.1%)	11 (14.7%)
RP Gleason Score	6	-	-	-	-	4 (13.8%)	4 (13.8%)
	7	-	-	-	-	18 (62.1%)	18 (62.1%)
	8+	-	-	-	-	7 (24.1%)	7 (24.1%)
D'Amico Risk	Low	-	10 (90.9%)	11 (64.7%)	24 (52.2%)	6 (20.7%)	51 (49.5%)
	Intermediate	-	1 (9.1%)	3 (17.6%)	17 (36.9%)	16 (55.2%)	37 (35.9%)
	High	-	-	3 (17.6%)	5 (10.9%)	7 (24.1%)	15 (14.6%)

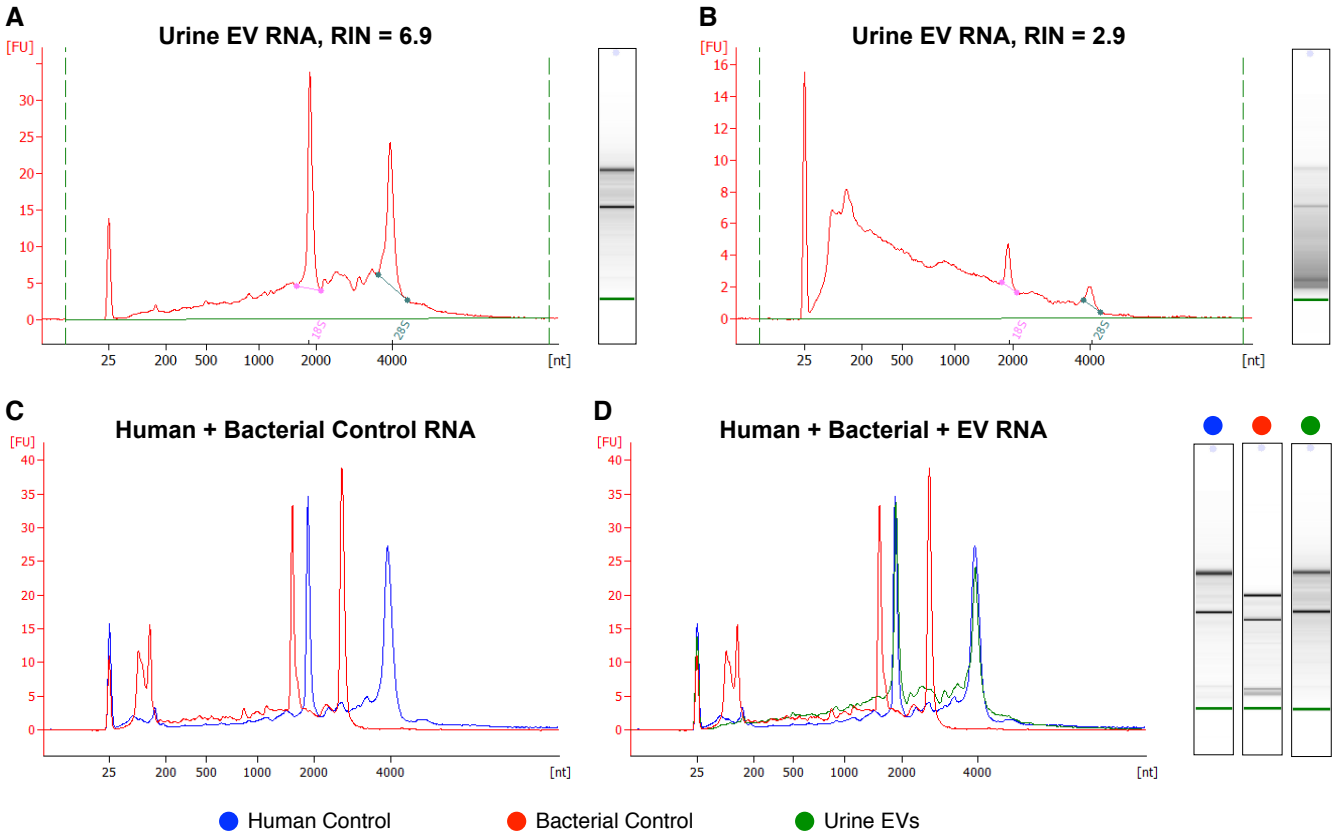
N.E.D., No Evidence of Disease; RP, Radical Prostatectomy; PSA, Serum Prostate Specific Antigen; SD, Standard Deviation; *Serum PSA values were not available for 8 of the 34 N.E.D. patients, mean ± SD is shown for 26 patients.

SUPPLEMENTARY MATERIAL – Detection of Prostate Cancer-Specific Transcripts in Extracellular Vesicles Isolated from Post-DRE Urine



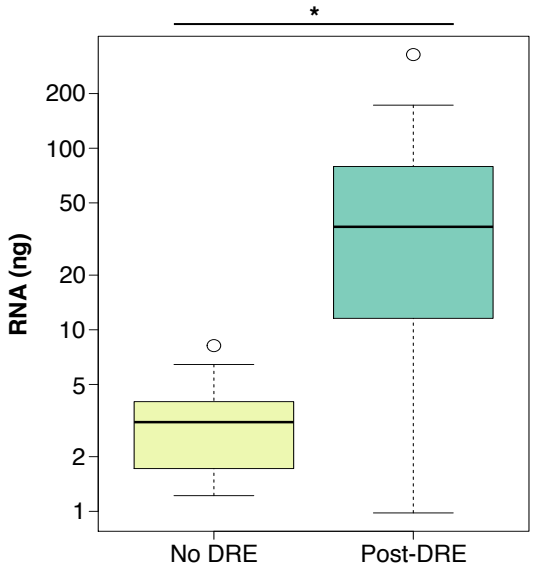
Supplementary Figure 1. Comparison of EV RNA yield when specimens are frozen prior to filtration. (A) Urine specimens were either processed to recover EVs on the day of collection (Same Day Filtration) or the supernatant was frozen so that ultrafiltration could be conducted at a later time (Post-Freeze Filtration). (B) The RNA yields from each of these methods are summarized with boxplots (n = 82 and 23 for Same Day Filtration and Post-Freeze Filtration, respectively; p = 0.667).

SUPPLEMENTARY MATERIAL – Detection of Prostate Cancer-Specific Transcripts in Extracellular Vesicles Isolated from Post-DRE Urine



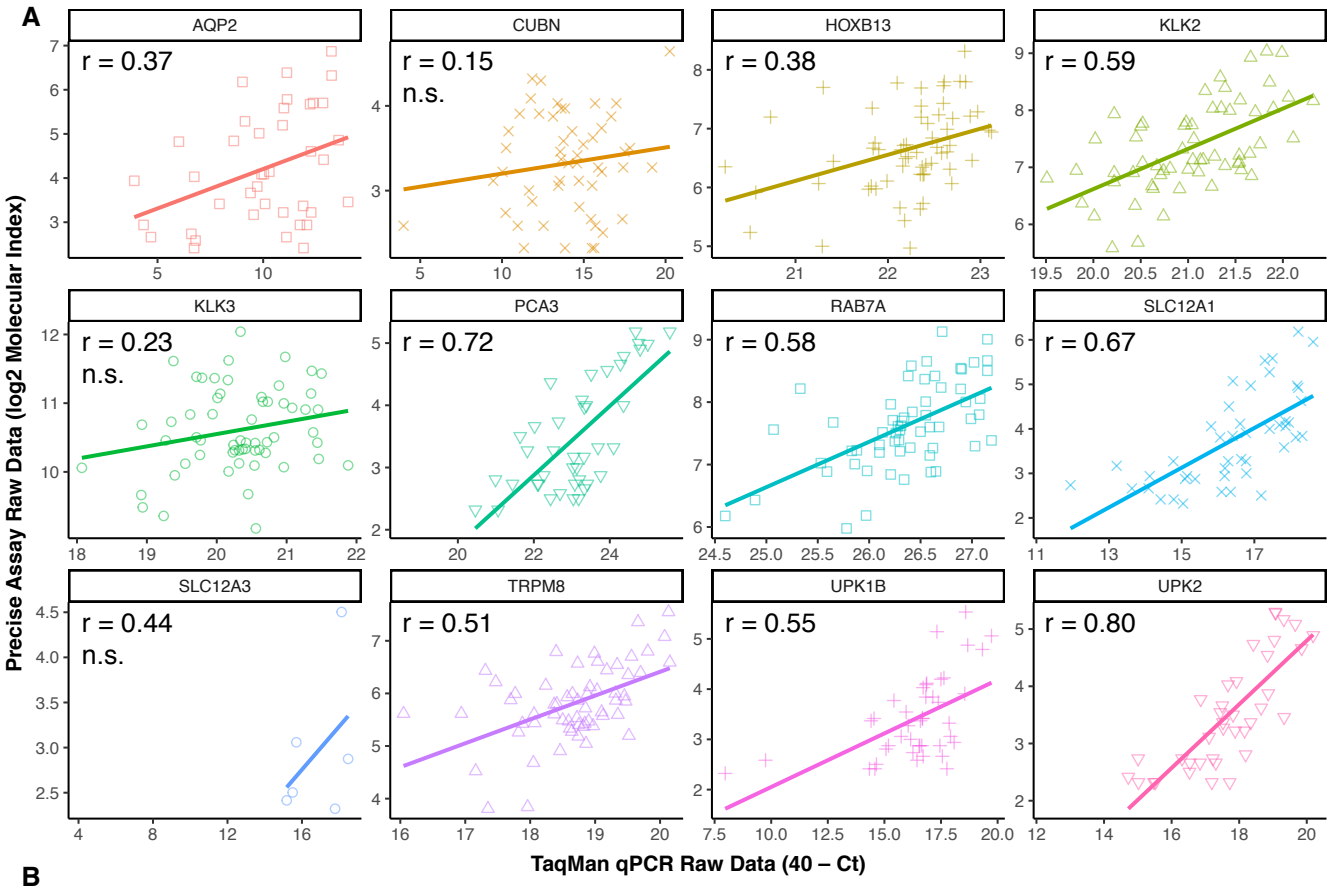
Supplementary Figure 2. Representative Bioanalyzer traces of RNA isolated from post-DRE urine. The Agilent RNA Pico RNA Assay and Bioanalyzer were used to determine the concentration and quality of RNA extracted from urine extracellular vesicles (EVs) and urine cell pellets. Bioanalyzer electropherograms and gel images representative of (A) high, and (B) low quality RNA from EVs are shown. (C) Comparison of human (blue) and bacterial (red) control RNAs demonstrated the characteristic 18S/28S eukaryotic rRNA peaks and 16S/23S prokaryotic rRNA peaks. (D) The EV sample (green) rRNA peaks overlap with the eukaryotic (blue) 18S/28S rRNAs and show no evidence of prokaryotic (red) rRNA peaks.

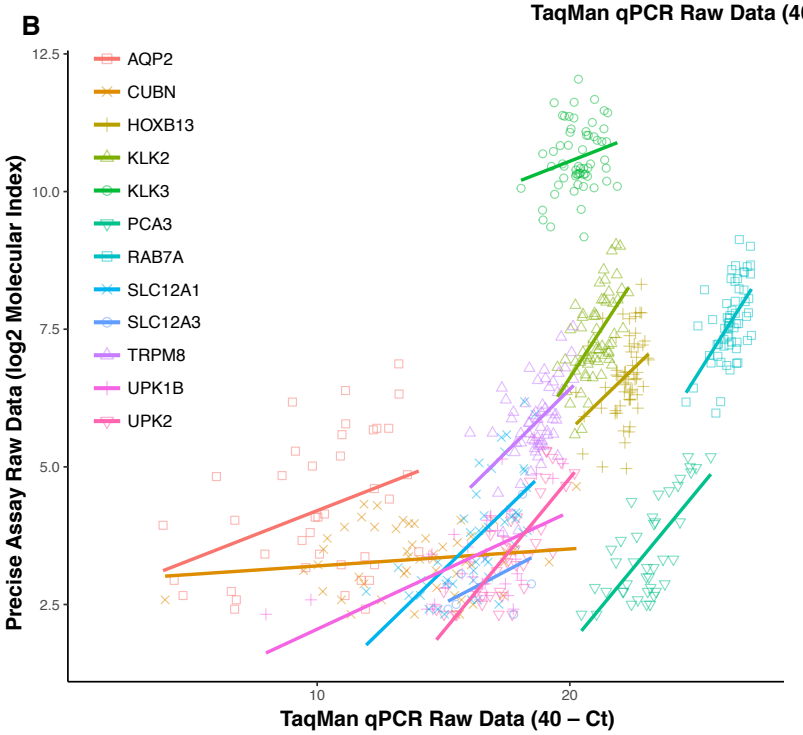
SUPPLEMENTARY MATERIAL – Detection of Prostate Cancer-Specific Transcripts in Extracellular Vesicles Isolated from Post-DRE Urine



Supplementary Figure 3. Effect of DRE on urine EV RNA yield. Urine specimens were collected from men that provided urine with (Post-DRE) or without (No DRE) a DRE prior to specimen collection. The RNA yields from each of these groups are summarized and shown on a logarithmic scale (n = 14 and 22 for No DRE and Post-DRE, respectively; $p < 0.001$).

SUPPLEMENTARY MATERIAL – Detection of Prostate Cancer-Specific Transcripts in Extracellular Vesicles Isolated from Post-DRE Urine





Supplementary Figure 4. Analysis of TaqMan qPCR and Precise Assay correlation. Each transcript was assessed by TaqMan qPCR, and Precise Assay. The TaqMan assay values are shown as 40 minus cycle threshold (with the threshold set to 0.1 for all assays). The Precise Assay data is shown as log2 molecular index values. Undetected values were excluded from plotting and correlation analysis (n = 60, except *AQP2* (40), *CUBN* (52), *PCA3* (41), *SLC12A1* (45), *SLC12A3* (6), *UPK1B* (43), and *UPK2* (38)). *ERG*, *NPHS2*, *PTH1R*, and *SLC34A1* were not detected in any sample by the Precise Assay and are not shown. (A) Individual plots for each gene were created. Pearson's correlation analysis was used to determine the degree of concordance between the two assays (r values shown). All correlations were significant (adjusted $p < 0.05$) except for *CUBN*, *KLK3*, and *SLC12A3*. (B) The data from A has been combined onto a single plot to demonstrate the relationships between all of the genes assessed.

SUPPLEMENTARY MATERIAL – Detection of Prostate Cancer-Specific Transcripts in Extracellular Vesicles Isolated from Post-DRE Urine

Supplementary Table 1. TaqMan Assay IDs

GENE	TAQMAN ASSAY ID
AQP2	Hs00166640_m1
CUBN	Hs00153607_m1
ERG	Hs01554629_m1
HOXB13	Hs00197189_m1
KLK2	Hs00428384_g1
KLK3	Hs02576345_m1
NPHS2	Hs00387817_m1
PCA3	Hs01371939_g1
PTH1R	Hs00174895_m1
RAB7A	Hs01115139_m1
SLC12A1	Hs00165731_m1
SLC12A3	Hs01027568_m1
SLC34A1	Hs01092910_m1
TRPM8	Hs01066596_m1
UPK1B	Hs01041715_m1
UPK2	Hs00171854_m1