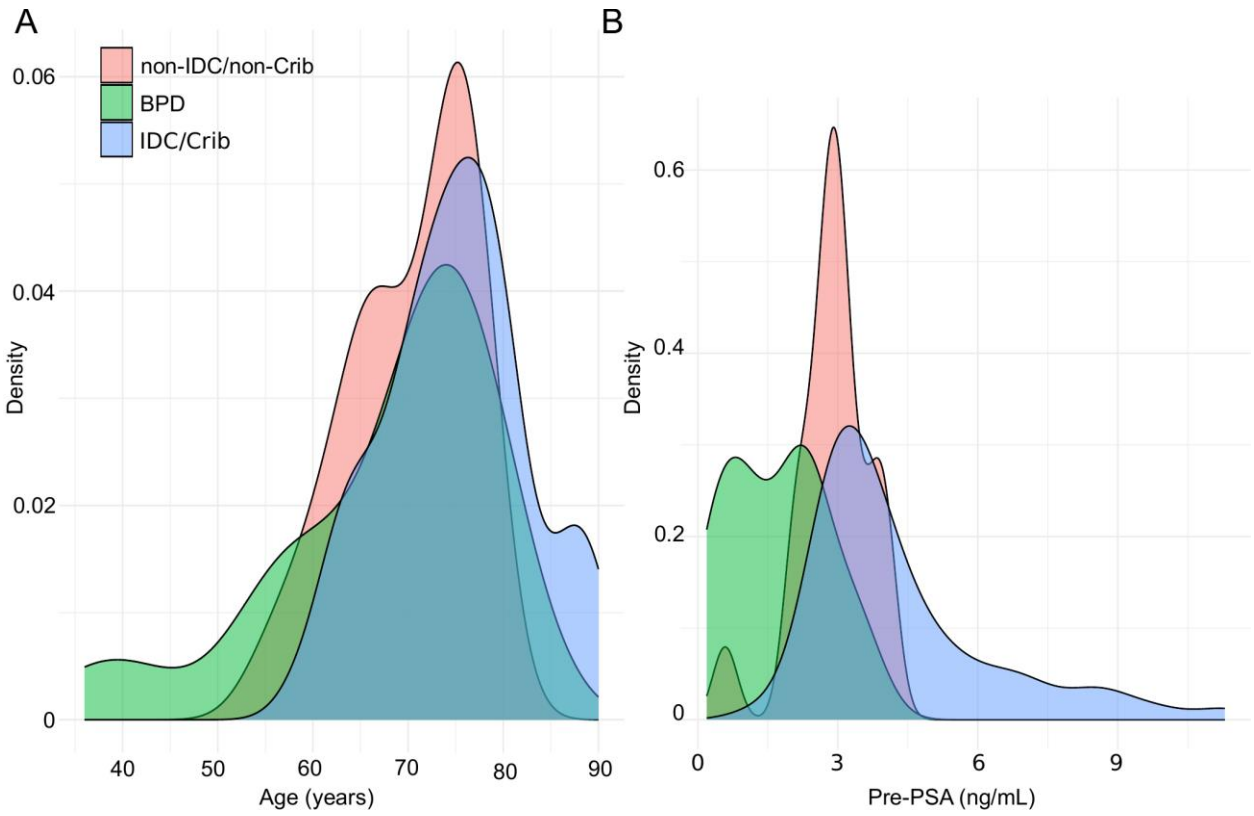


Figure S1. Density distribution of age and pre-PSA across main clinical subgroups.

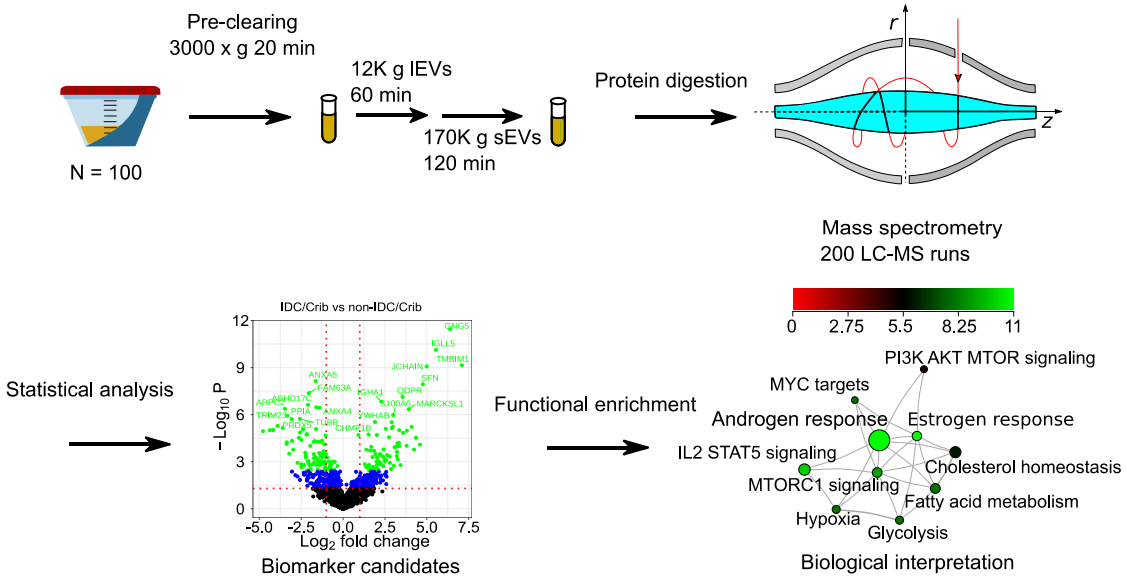


Figure S2. *Workflow for analysis of urinary small extracellular vesicles (sEVs).*

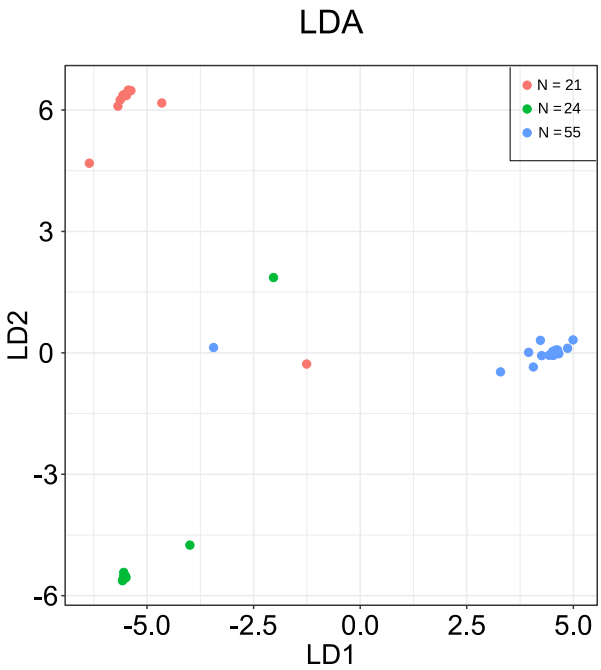


Figure S3. *Linear Discriminant Analysis (LDA) of three groups: BPD (green), non-IDC/non-Crib (red), and IDC/Crib (blue).*

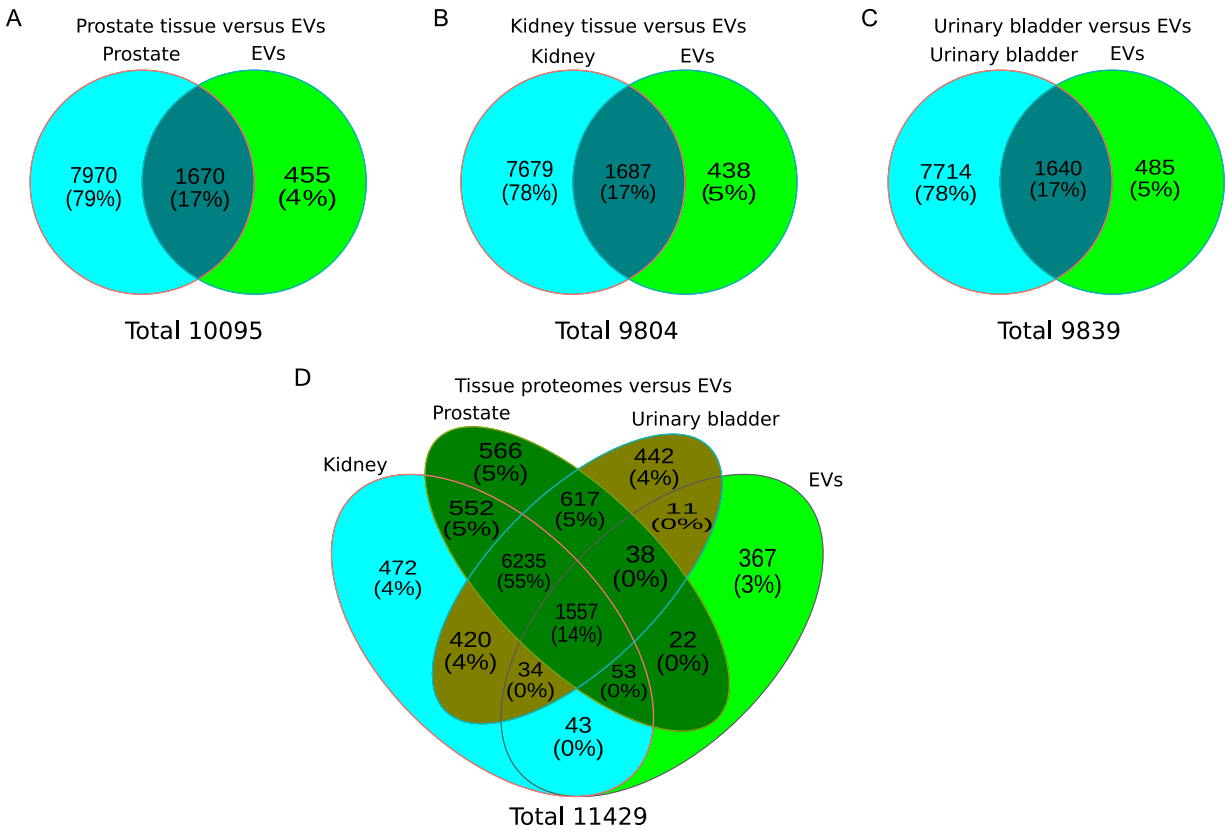


Figure S4. Venn diagrams visualizing prostate, kidney and bladder tissue proteome overlap with small urinary EV proteome. Pairwise comparison of small urinary EV proteome with A) prostate tissue, B) kidney tissue and C) urinary bladder tissue. D) Direct comparison of all three tissues with small urinary EV proteome.

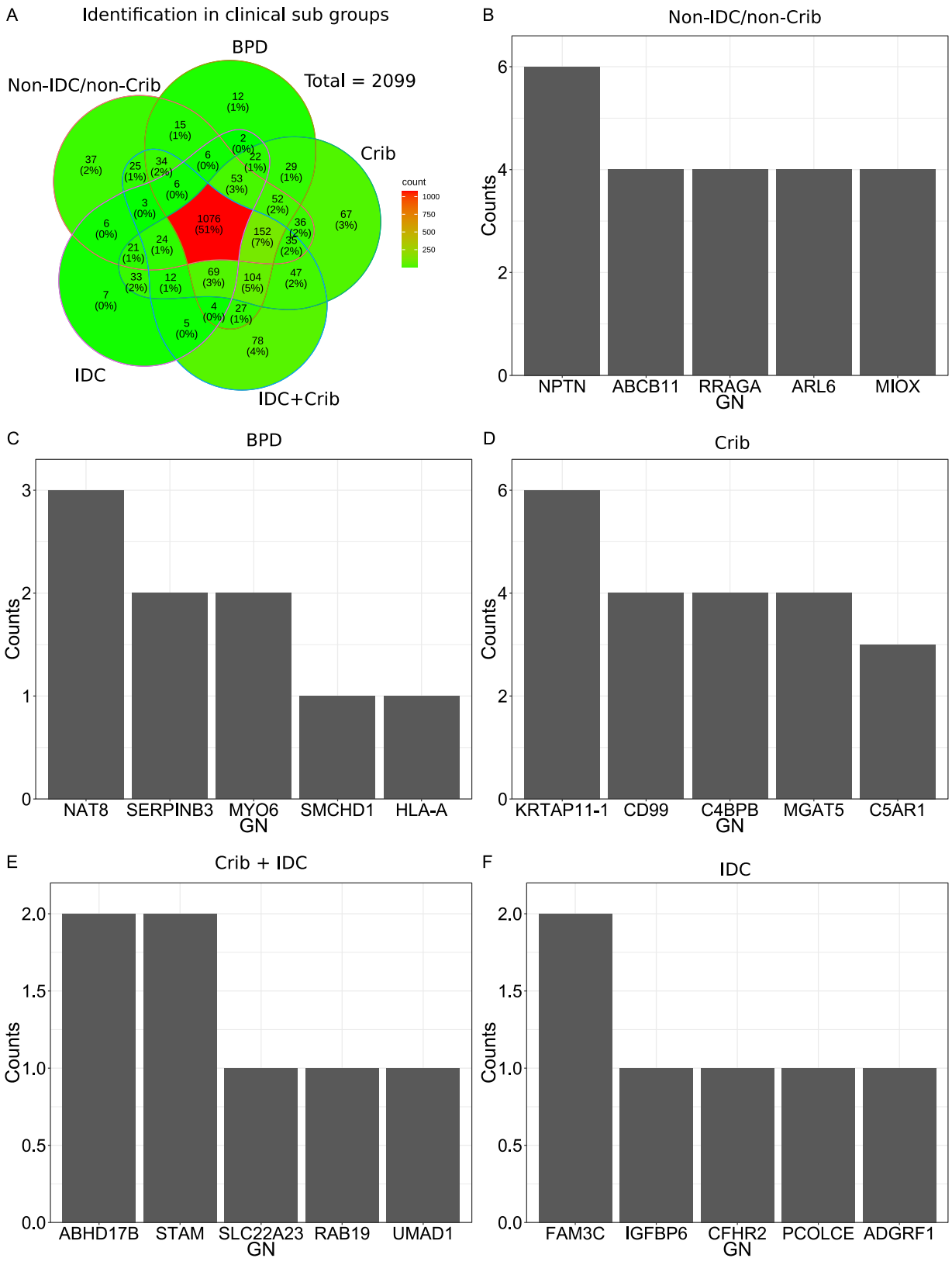
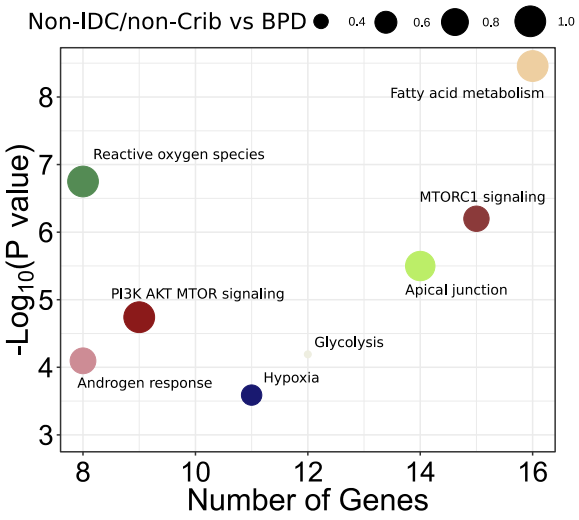
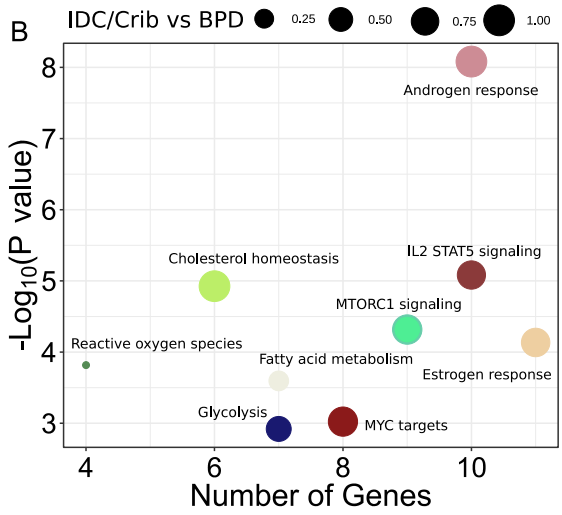

A
Identification in clinical sub groups
BPD
Non-IDC/non-Crib
Total = 2099
Crib
IDC
IDC+Crib
count
1000
750
500
250
12 (1%)
15 (1%)
2 (0%)
6 (0%)
22 (1%)
29 (1%)
37 (2%)
25 (1%)
34 (2%)
53 (3%)
6 (0%)
3 (0%)
52 (2%)
67 (3%)
1076 (51%)
6 (0%)
152 (7%)
36 (2%)
24 (1%)
35 (2%)
21 (1%)
33 (2%)
12 (1%)
69 (3%)
104 (5%)
47 (2%)
7 (0%)
4 (0%)
27 (1%)
5 (0%)
78 (4%)
B
Non-IDC/non-Crib
Counts
NPTN
ABCB11
RRAGA
ARL6
MIOX
GN
C
BPD
Counts
NAT8
SERPINB3
MYO6
SMCHD1
HLA-A
GN
D
Crib
Counts
KRTAP11-1
CD99
C4BPB
MGAT5
C5AR1
GN
E
Crib + IDC
Counts
ABHD17B
STAM
SLC22A23
RAB19
UMAD1
GN
F
IDC
Counts
FAM3C
IGFBP6
CFHR2
PCOLCE
ADGRF1
GN

Figure S5. *Overview of proteins identified uniquely in clinical subgroups. A) Venn diagram of overlapping identified proteins in each clinical subgroup. Box plots of the five most consistently reported proteins that are unique for each clinical subgroup B) non-IDC/non-Crib, C) BPD, D) Crib, F) Crib+IDC and G) IDC.*

A


Non-IDC/non-Crib vs BPD
0.4
0.6
0.8
1.0
Fatty acid metabolism
Reactive oxygen species
MTORC1 signaling
Apical junction
PI3K AKT MTOR signaling
Glycolysis
Androgen response
Hypoxia
-Log10(P value)
Number of Genes

B


IDC/Crib vs BPD
0.25
0.50
0.75
1.00
Androgen response
Cholesterol homeostasis
IL2 STAT5 signaling
MTORC1 signaling
Reactive oxygen species
Fatty acid metabolism
Estrogen response
Glycolysis
MYC targets
-Log10(P value)
Number of Genes

C

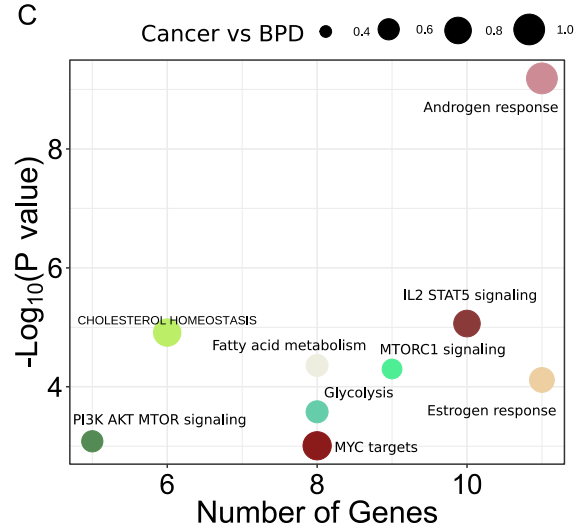

Cancer vs BPD
0.4
0.6
0.8
1.0
Androgen response
IL2 STAT5 signaling
CHOLESTEROL HOMEOSTASIS
Fatty acid metabolism
MTORC1 signaling
Glycolysis
Estrogen response
PI3K AKT MTOR signaling
MYC targets
-Log10(P value)
Number of Genes

Figure S6. *Functional enrichment analysis of cancer hallmark proteins based on significant regulated proteins for pairwise comparisons to control. Scatter plot displaying -log$_{10}$ P value for enrichment as a function of the number of proteins matching for each functional group based on the pairwise comparisons A) non-IDC/non-Crib vs BPD, B) IDC/Crib vs BPD and C) cancer vs BPD.*

MISEV 2018 check list

1-Nomenclature
Mandatory
+++ Generic term extracellular vesicle (EV): With demonstration of extracellular (no intact cells) and vesicular nature per these characterization (Section 4) and function (Section 5) guidelines
OR
+++ Generic term, e.g., extracellular particle (EP): no intact cells but MISEV guidelines not satisfied Encouraged (choose one)
+ Generic term extracellular vesicle (EV) + specification
(size, density, other)
+ Specific term for subcellular origin: e.g., ectosome, microparticle, microvesicle (from plasma membrane), exosome (from endosomes), with demonstration of the
subcellular origin
+ Other specific term: with definition of specific criteria

We applied the generic term extracellular vesicle (EV) throughout the manuscript.

2-Collection and pre-processing
++ Donor status if available (age, sex, food/water intake, collection time, disease, medication, other)
+++ Volume of biofluid or volume/mass of tissue sample collected per donor
++ Total volume/mass used for EV isolation (if pooled from several donors)
+++ All known collection conditions, including additives, at time of collection
+++ Pre-treatment to separate major fluid-specific contaminants before EV isolation
+++ Temperature and time of biofluid/tissue handling before and during pre-treatment

+++ Storage and recovery (e.g., thawing) of CCM, biofluid, or tissue before EV isolation (storage temperature, vessel, time; method of thawing or other sample preparation)
+++ Storage and recovery of EVs after isolation (temperature, vessel, time, additive(s)...)

Mid-stream urine (30 – 150 mL) from PCa suspects were collected, immediately frozen at -80°C and stored upon collection until EV isolation. The time length from urine collection and EV isolation varied between 2 and 6 months. Frozen stored urine was thawed at room temperature, followed by consecutive increased centrifugation speed. Isolated EVs were resuspended in PBS filtered with 0.2µm filter and stored in low binding protein microcentrifuge tubes at -80°C.

3-EV separation and concentration

Experimental details of the method

++ Centrifugation: reference number of tube(s), rotor(s), adjusted k factor(s) of each centrifugation step (= time+ speed+ rotor, volume/density of centrifugation conditions), temperature, brake settings

++ Filtration: reference of filter type (=nature of membrane, pore size...), time and speed of centrifugation, volume before/after (in case of concentration)

++ Antibody-based : reference of antibodies, mass Ab/ amount of EVs, nature of Ab carrier (bead, surface) and amount of Ab/carrier surface

++ Other...: all necessary details to allow replication

++ Additional step(s) to concentrate, if any

++ Additional step(s) to wash matrix and/or sample, if any Specify category of the chosen EV separation/concentration method (Table 1):

+ High recovery, low specificity = mixed EVs and non-EV components OR

+ Intermediate recovery, intermediate specificity =mixed EVs with limited non-EV components OR

+ Low recovery, high specificity = subtype(s) of EVs with as little non-EV as possible OR

+ High recovery, high specificity = subtype(s) of EVs with as little non-EV as possible

Frozen urine specimens were thawed at room temperature centrifuged at 3000× g for 20 min at 4°C and then at 12,000 ×*g* for 60 min at 4°C using polypropylene centrifuge tubes (Beckman Coulter, cat. no. 326823). Clarified urine was ultracentrifuged in an Optima L-100XP ultracentrifuge (Beckman Coulter, Brea, CA, USA) at 170,000× g at 4°C for 120 min with a Type 32 Ti rotor (Beckman Coulter) to pellet EVs with acceleration and deacceleration at maximum. The supernatant was carefully removed, and crude EV-containing pellets were resuspended in ice-cold PBS and stored at -80°C until further analysis.

4-EV characterization

Quantification (Table 2a, Section 4-a)

+++ Volume of fluid, and/or cell number, and/or tissue mass used to isolate EVs

+++ Global quantification by at least 2 methods: protein amount, particle number, lipid amount, expressed per volume of initial fluid or number of producing cells/mass of tissue

+++ Ratio of the 2 quantification figures Global characterization (Section 4-b, Table 3)

+++ Transmembrane or GPI-anchored protein localized in cells at plasma membrane or endosomes

+++ Cytosolic protein with membrane-binding or -association capacity

+++ Assessment of presence/absence of expected contaminants (At least one each of the three categories above)
++ Presence of proteins associated with compartments other than plasma membrane or endosomes
++ Presence of soluble secreted proteins and their likely transmembrane ligands
+ Topology of the relevant functional components (Section 4-d) Single EV characterization (Section 4-c)
+++ Images of single EVs by wide-field and close-up: e.g. electron microscopy, scanning probe microscopy, super-resolution fluorescence microscopy
+++ Non-image-based method analysing large numbers of single EVs: NTA, TRPS, FCS, high-resolution flow cytometry, multi-angle light-scattering, Raman spectroscopy, etc.

Thirty-six milliliters from each patient sample were used to isolate EVs. Total protein EV preparations were quantified using BCA assay. Particle count and particle size analysis were performed using Nanotracking analysis by NanoSight NS300. The ratio of the number of particles per microgram of total EV protein was calculated. As for MISEV 2018 recommendations on protein content-based EV characterization, CD63 (Category 1) and ALIX (Category 2) demonstrated the presence of EVs. As for specificity of small EV subtypes (Category 4) we have used GRP75 and TOM20 as markers of transmembrane, lipid-bound and soluble proteins associated to other intracellular compartments than PM/endosomes. Detailed immunoblot methods are described in the methods section.

6-Reporting

+++ Submission of data (proteomic, sequencing, other) to relevant public, curated databases or open-access repositories
++ Temper EV-specific claims when MISEV requirements cannot be entirely satisfied (Section 6-b)

The mass spectrometry proteomics data that support the findings of this study have been deposited in ProteomeXchange Consortium via the PRIDE partner with the PXD043874 accession codes (DOI: 10.6019/PXD043874).

STROBE check list

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Profiling of urinary extracellular vesicle protein signatures from patients with cribriform and intraductal prostate carcinoma in a cross-sectional study	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
		p.1
Introduction		
Background/rationale	2	p.2
Objectives	3	p.2
Methods		
Study design	4	Present key elements of study design early in the paper: P. 3 top
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection: P. 3 top
Participants	6	*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants: P. 3 top
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. P.3
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group: P.3
Bias	9	Describe any efforts to address potential sources of bias. P.3
Study size	10	Explain how the study size was arrived at P.3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why. P.5
Statistical methods	12	Describe all statistical methods, including those used to control for confounding. P4-5
		(*b*) Describe any methods used to examine subgroups and interactions. P4-5
		(*c*) Explain how missing data were addressed. P.4-5
		(*e*) Describe any sensitivity analyses. P.5

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. **All patients that fulfilled the inclusion and exclusion criteria and provided informed consent were analysed.**
		(b) Give reasons for non-participation at each stage. NA
		(c) Consider use of a flow diagram. NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. P.3
		(b) Indicate number of participants with missing data for each variable of interest. P.6 Table 1.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure NA
		Cross-sectional study—Report numbers of outcome events or summary measures P.6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Table S1-S3)
		(*b*) Report category boundaries when continuous variables were categorized NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses P.5
Discussion		
Key results	18	Summarise key results with reference to study objectives P.18
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias P.18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results P.17
Other information		
Funding	22	P.19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.