Supporting information for:

Prostate-specific membrane antigen (PSMA) -positive extracellular vesicles in urine - a potential liquid biopsy strategy for prostate cancer diagnosis?

Susann Allelein[1]*, Keshia Aerchlimann[1], Gundula Rösch[2], Roxana Khajehamiri[2], Andreas Kölsch[1], Christian Freese[2], Dirk Kuhlmeier[1]

[1] Affiliation: Fraunhofer Institute for Cell Therapy and Immunology (IZI) Leipzig, Germany
[2] Affiliation: Fraunhofer Institute for Microengineering and Microsystems (IMM) Mainz, Germany
* Correspondence: susann.allelein@izi.fraunhofer.de;

Keywords: extracellular vesicles, prostate specific membrane antigen, microarray, immunomagnetic isolation, automated, prostate cancer

Table S1. Cohort characteristics of PCa patients and benign male controls. The risk group corresponds to the Gleason Score (GS) determined after prostate tissue biopsy.

RISK	#	AGE	PSA [ng/mL]	CREATININE [mmol/L]	GS	PROSTATE V [mL]
benign	1	56	14.11	23.99		126
benign	2	53	20.20	14.04		63
benign	3	66	3.89	2.55		70
benign	4	67	2.99	0.38		100
benign	5	78	0.95	0.62		24
benign	6	74	3.50	2.45		87
benign	7	74	6.00	7.03		50
benign	8	70	10.00	5.08		90
benign	9	66	2.25	20.17		40
benign	10	68	7.40	11.75		120
benign	11	58	11.70	18.31		70
benign	12	72	0.93	2.25		130
benign	13	56	8.00	32.66		70
benign	14	66	5.05	30.77		51
benign	15	78	5.50	7.78		75
benign	16	68	17.70	4.70	6	128
low	1	66	9.79	5.34		110
low	2	54	1.10	6.33		60
low	3	77	2.60	5.75		50
low	4	56	5.00	15.94	6	30
low	5	62	7.00	6.48	6	30
intermediate	1	55	12.20	14.21	7a	25
intermediate	2	68	5.86	13.97	7a	70
intermediate	3	67	12.60	7.26	7a	120

intermediate	4	63	12.90	8.99	6	60
intermediate	5	77	7.67	13.66	7a	30
intermediate	6	56	4.80	18.46	7	40
intermediate	7	75	12.70	20.40	7	15
intermediate	8	73	4.20	10.77	7	18
intermediate	9	78	6.10	10.78	7	10
intermediate	10	74	5.00	8.92	7	35
intermediate	11	63	5.12	15.59	7	40
intermediate	13	62	5.29	7.97	7	66
intermediate	14	76	8.44	8.86	7	45
intermediate	15	63	14.50	6.36	7	55
high	1	58	6.60	20.48	7	25
high	2	67	7.30	3.9	8	94
high	3	71	6.80	15.58	9	60
high	4	62	6.71	3.95	9	30
high	5	69	34.50	3.01	9	240
high	6	71	5.40	1.21	9	20
high	7	67	28.00	1.92	8	77

Table S2. Cohort characteristics of healthy female and male controls.

#	SEX	#	AGE	CREATININE [µmol/L]
1	female	1	35	24850
2	female	2	27	24730
3	female	3	30	1603
4	female	4	34	1963
5	female	5	32	1176
6	male	6	22	2724
7	male	7	36	2552
8	male	8	29	3950
9	male	9	29	21782
10	male	10	29	16070

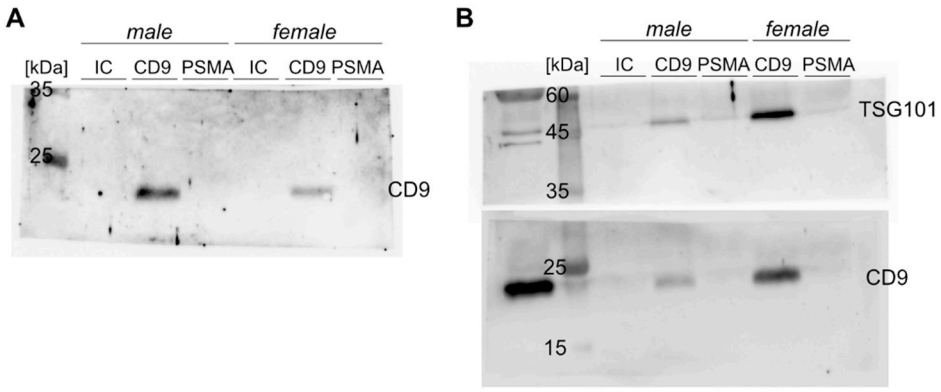

Figure S1. Specific uEV isolation using immunomagnetic beads. Western blot analysis for CD9 and/or TSG101 of CD9- or PSMA-positive uEVs and the isotype control (IC) from female and male intermediate PCa risk urine from patient intermediate 15 (**A**) and 3 (**B**).

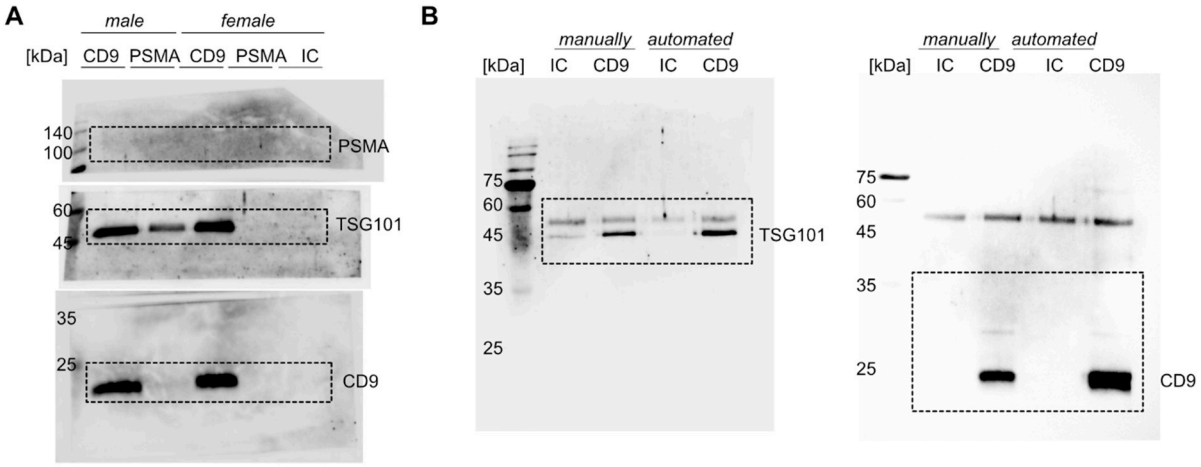

Figure S2. Western blot images for specific uEV isolation using immunomagnetic beads with cropped areas indicated by dashed lines used in figure 2. Antibody incubation on membrane cuts according to the molecular weight of the investigated protein.

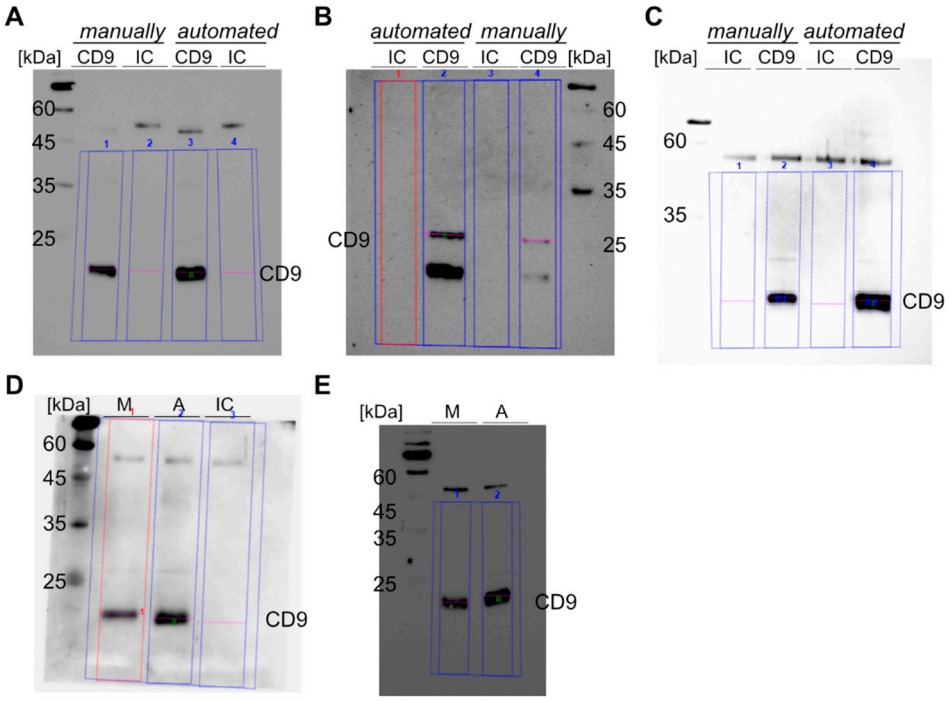

Figure S3. Western blot analysis of CD9 from specific uEV immunomagnetic isolation by manually (M) and automated (A) performed procedure targeting the isotype control (IC) or CD9 (**A** -**C**) or a mix of CD63 and CD81 (**D**, **E**) from 5 mL of cell-free urine. Relative band intensities of CD9 analyzed in Image Lab software (Bio-Rad Laboratories) and summarized in Fig. 2.

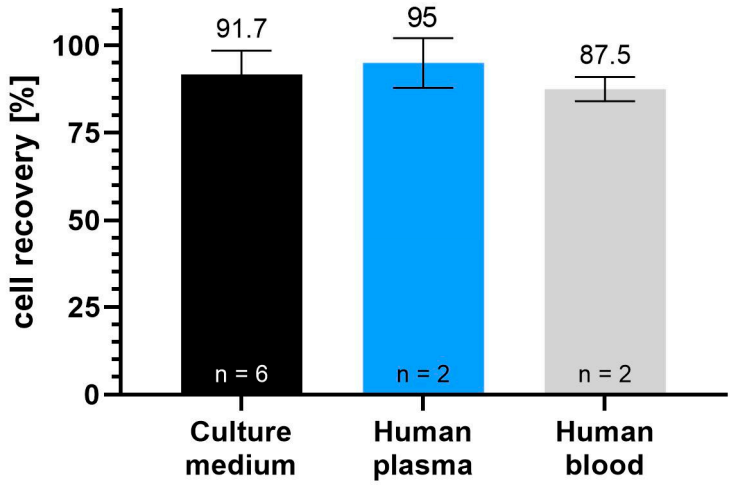

Figure S4. Characterization of the IsoMAG-ONE10.0 isolation system. For testing the functionality and the optimal isolation parameters, such as number of washing steps, buffers used, mixing times and volumes, enrichment of cells from cell culture medium, human plasma and whole blood was performed. For this purpose, 20 fluorescently stained cells were added to the enrichment medium and the cells were automatically enriched with EpCAM-coated Dynabeads. After enrichment, the cell number of the enriched cells was determined and the percentages calculated in relation to the 20 previously added cells. This protocol did not only show best enrichment properties but also lowest loss of beads during the process which is of importance for the isolation of EVs as demonstrated in Fig. 3.

Table S3. Assay parameters for IsoMAG-ONE10.0 (duration: 1:34 h)

No.	Step	Description
1	Reset all axes	Required
2	Take pipette	Take 10ml pipette
3	Move to well 1	5ml + 100 µl *Beads* in 6ml tube
4	Prepare pipette	Pump 0,5ml air
5	Dive in pipette	Dive in fluid
6	Mixing	30min; 20ml/min
7	Get *Beads*	Magnet to pipette and release fluid; 60s
8	Move to well 2	4ml in 6ml tube
9	Prepare pipette	Pump 0,5ml air
10	Dive in pipette	Dive in fluid
11	Peel off	10x; 20ml/min; 1,1ml
12	Washing	20x ; 20ml/min
13	Get *Beads*	Magnet to pipette and release fluid; 60s
14	Move to well 3	4ml in 6ml tube
15	Prepare pipette	Pump 0,5ml air
16	Dive in pipette	Dive in fluid
17	Peel off	10x; 20ml/min; 1,1ml
18	Washing	20x ; 20ml/min
19	Get *Beads*	Magnet to pipette and release fluid; 60s
20	Move to well 4	1ml in 2ml tube
21	Prepare pipette	Pump 0,5ml air

22	Dive in pipette	Dive in fluid
23	Washing	25x ; 20ml/min
24	Release Fluid	Release fluid from pipette without magnet
25	Drop pipette	Drop 10ml pipette
26	Take pipette	Take 1,25ml pipette
27	Move to well 4	1ml in 2ml tube
28	Prepare pipette	Pump 0,5ml air
29	Dive in pipette	Dive in fluid
30	Mixing	5x; 5ml/min
31	Get *Beads*	Magnet to pipette and release fluid; 60s
32	Move to well 5	0,2ml in 1,9 ml tube
33	Prepare pipette	Pump 0,5ml air
34	Dive in pipette	Dive in fluid
35	Washing	30x ; 2 ml/min
36	Release Fluid	Release fluid from pipette without magnet
37	Drop pipette	Drop 1,25ml pipette