OMTM, Volume *22*

Supplemental information

Unique somatic variants in DNA from urine exosomes of individuals with bladder cancer

Xunian Zhou, Paul Kurywchak, Kerri Wolf-Dennen, Sara P.Y. Che, Dinanath Sulakhe, Mark D'Souza, Bingqing Xie, Natalia Maltsev, T. Conrad Gilliam, Chia-Chin Wu, Kathleen M. McAndrews, Valerie S. LeBleu, David J. McConkey, Olga V. Volpert, Shanna M. Pretzsch, Bogdan A. Czerniak, Colin P. Dinney, and Raghu Kalluri

Supplementary Material

Unique somatic variants in the DNA from urine exosomes of bladder cancer patients

Xunian Zhou[1,‡], Paul Kurywchak[1,‡], Kerri Wolf-Dennen[1], Sara P.Y. Che[1], Dinanath Sulakhe[2], Mark D'Souza[2], Bingqing Xie[2], Natalia Maltsev[2], T. Conrad Gilliam[2], Chia-Chin Wu[3], Kathleen M. McAndrews[1], Valerie S. LeBleu[1,4], David J McConkey[5], Olga Volpert[1], Shanna Pretzsch[6], Bogdan A Czerniak[7], Colin Dinney[6], Raghu Kalluri[1,8,9,*]

[1]Department of Cancer Biology, University of Texas MD Anderson Cancer Center, Houston, TX

[2]Department of Human Genetics, University of Chicago, Chicago, IL

[3]Department of Genomic Medicine, University of Texas MD Anderson Cancer Center, Houston, TX

[4]Feinberg School of Medicine, Northwestern University, Chicago, IL

[5]Johns Hopkins Greenberg Bladder Cancer Institute, Baltimore, MD

[6]Department of Urology, University of Texas MD Anderson Cancer Center, Houston, TX

[7]Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, TX

[8]School of Bioengineering, Rice University, Houston, TX

[9]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX

[‡]These authors contributed equally

*Corresponding author:

Raghu Kalluri, MD, PhD

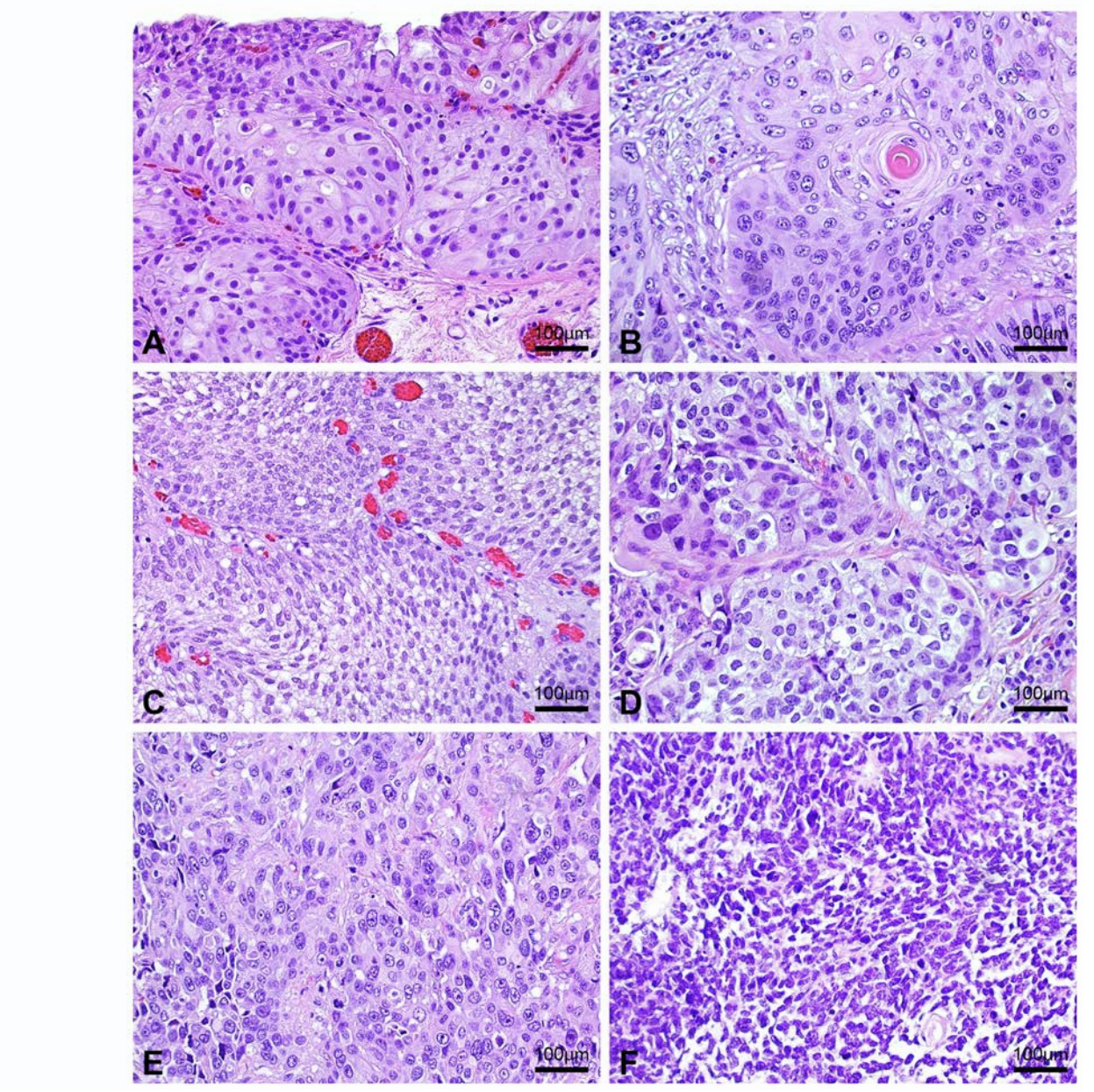
Email: rkalluri@mdanderson.org

Supplementary data with analyses for individual patients

https://doi:10.17632/kg2ccb425s.1

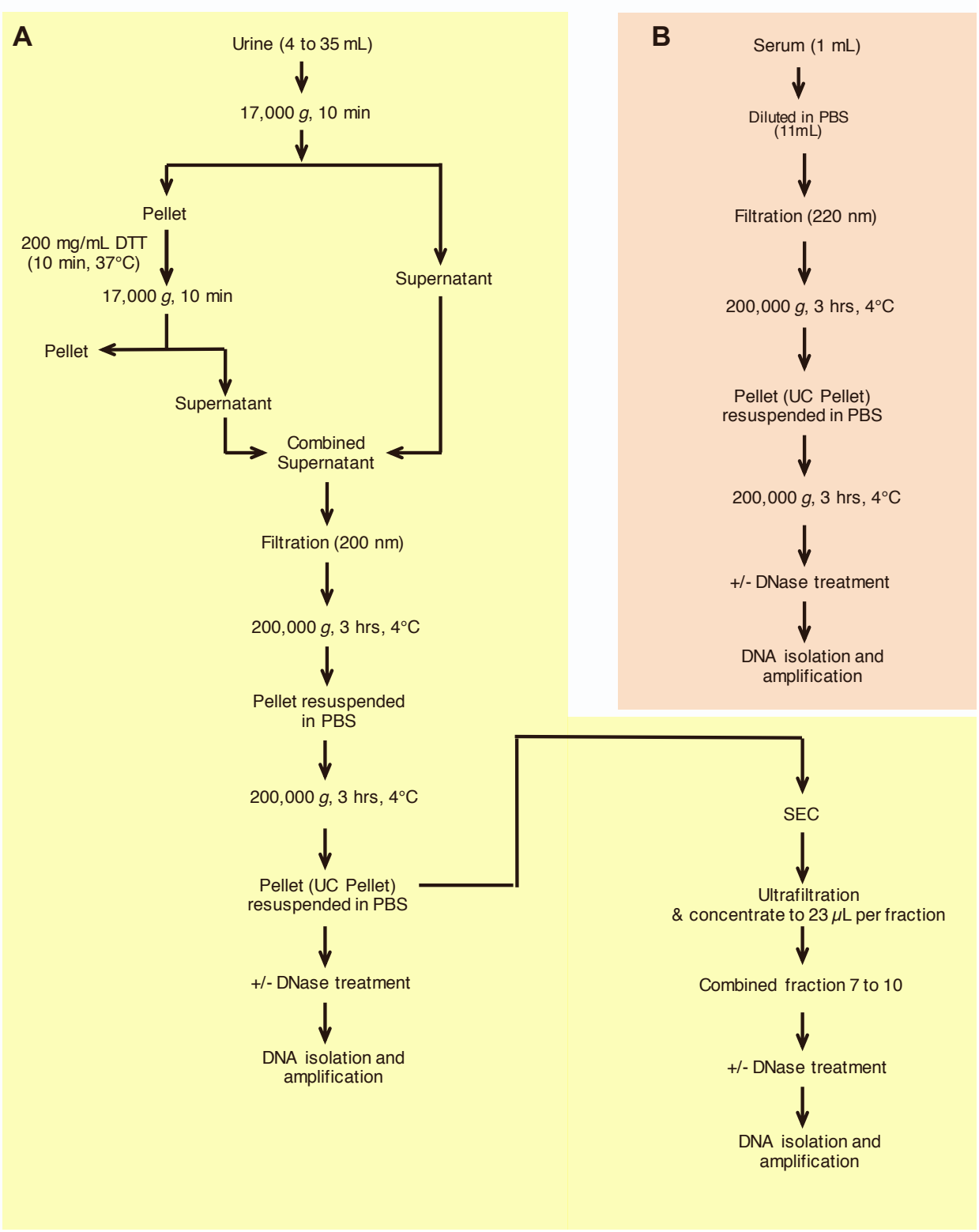
The files contain (a) annotations of the genes containing somatic variations; (b) the results of the enrichment analyses for the identification of the functional categories and pathways over-represented in the set of genes containing somatic variations; (c) the reconstructions of the molecular networks of genes identified in every sample (e.g. tumor or urine), and (d) the predictions of the miRNAs potentially targeting the UTRs containing somatic variations.

Supplementary Figures



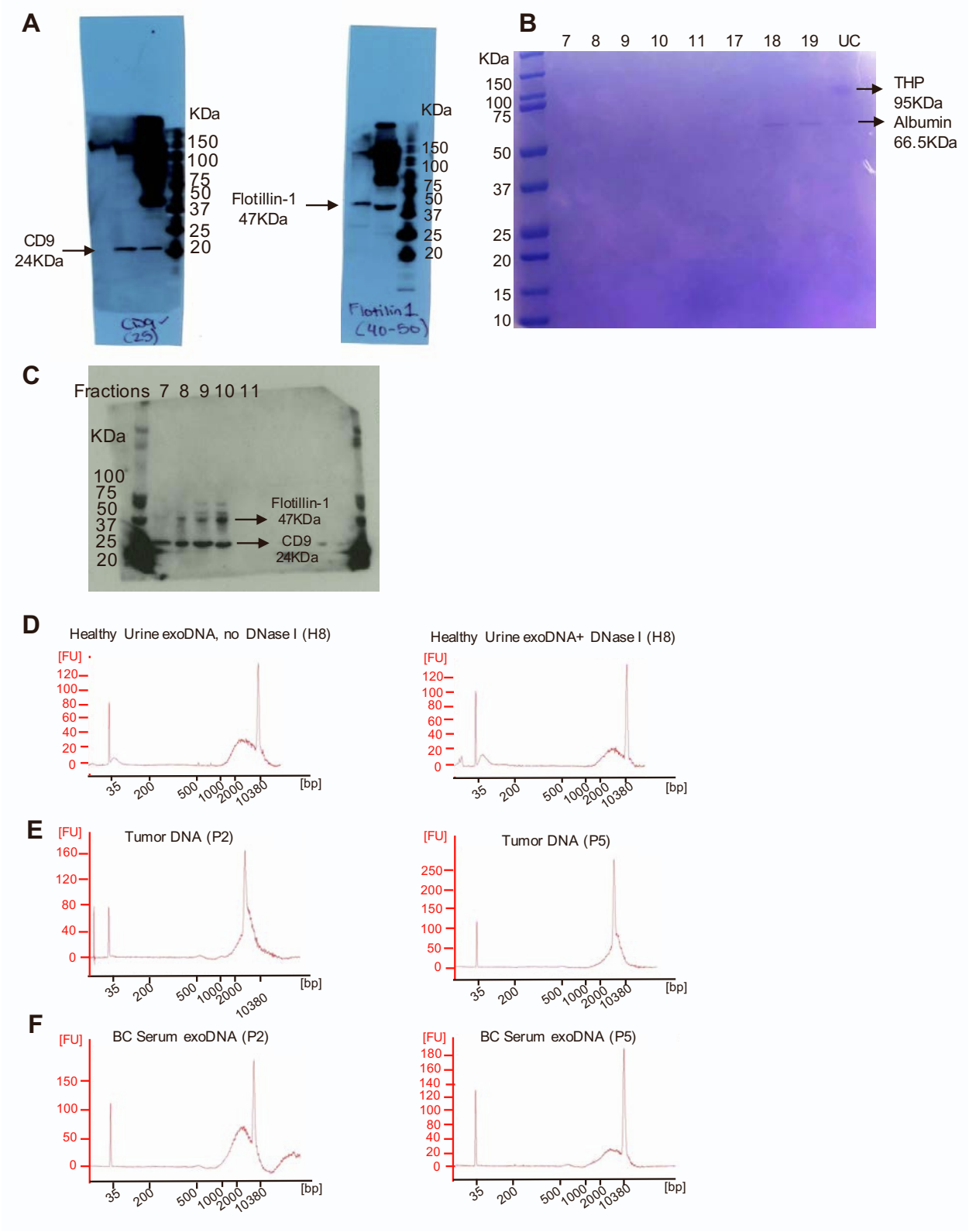
Supplementary Figure 1. Representative H&E images of bladder cancer patients

Representative H&E images of the indicated bladder cancer patient; please see **Supplementary Table 1** for details. (**A**) Papillary urothelial carcinoma, low grade (P1). (**B**) Urothelial carcinoma with squamous differentiation, high grade (P2). (**C**) Papillary urothelial carcinoma, low grade (P3). (**D**) Urothelial carcinoma, high grade (P4). (**E**) Urothelial carcinoma, high grade (P5). (**F**) Small cell variant of urothelial carcinoma (P6). Scale bar, 100 μm.



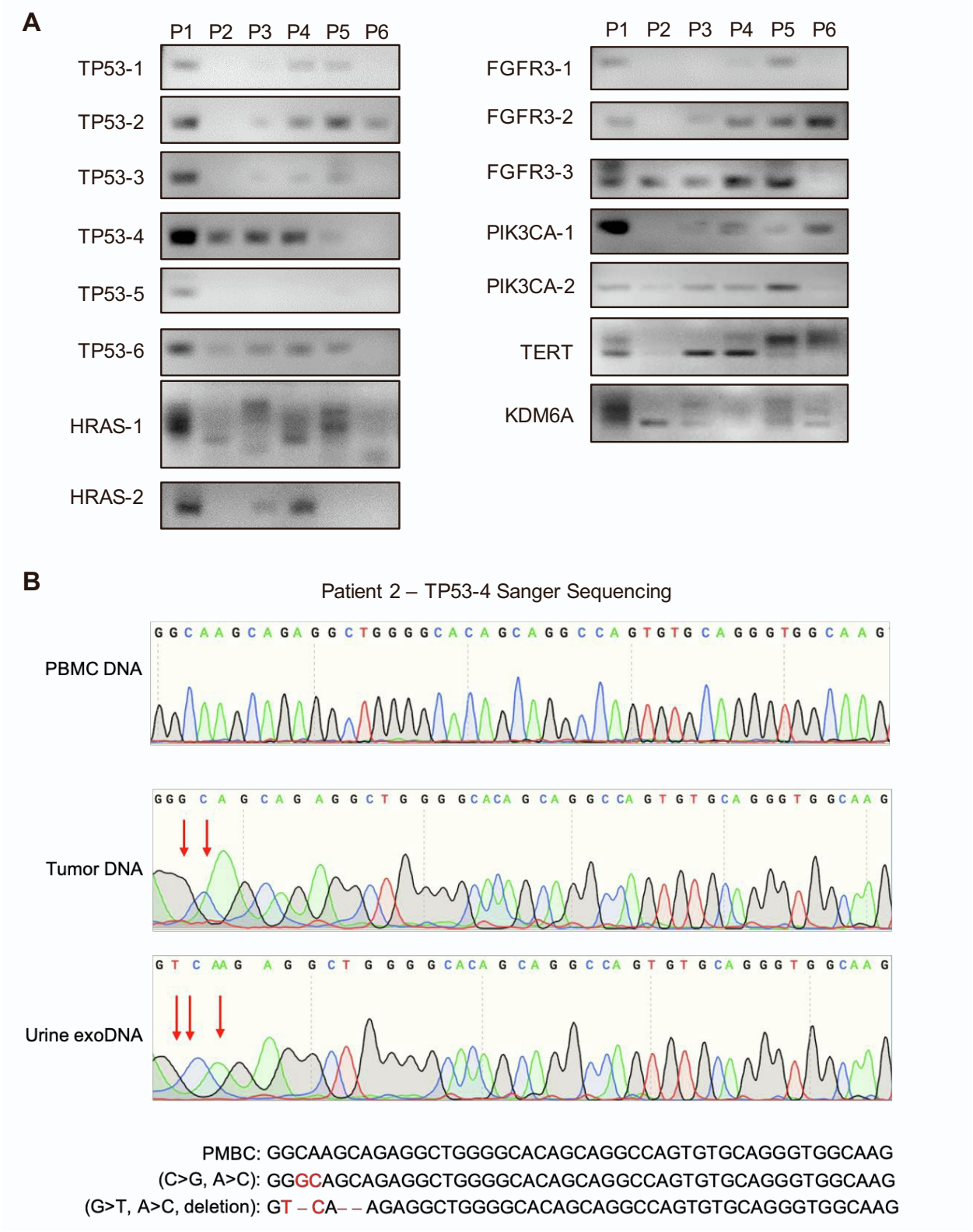
Supplementary Figure 2. Diagram of urine exosome isolation and purification procedure. Healthy sample and BC patient urine are thawed from -80°C storage and centrifuged to remove protein contaminants

and other debris. The resulting pellet contains Tamm-Horsfall (THS) glycoprotein, which is known to trap exosomes. These exosomes can be liberated by digesting Tamm-Horsfall in DTT. Liberated exosomes, now in the supernatant following an additional spin, are combined with the supernatant of the first spin. The combined supernatants are then processed using filtration and ultracentrifugation (UC) to collect exosomes for downstream analysis. The exosomes from UC (UC pellets) were also subjected to size exclusion chromatography and fractions 7-10 were pooled for further experiments.



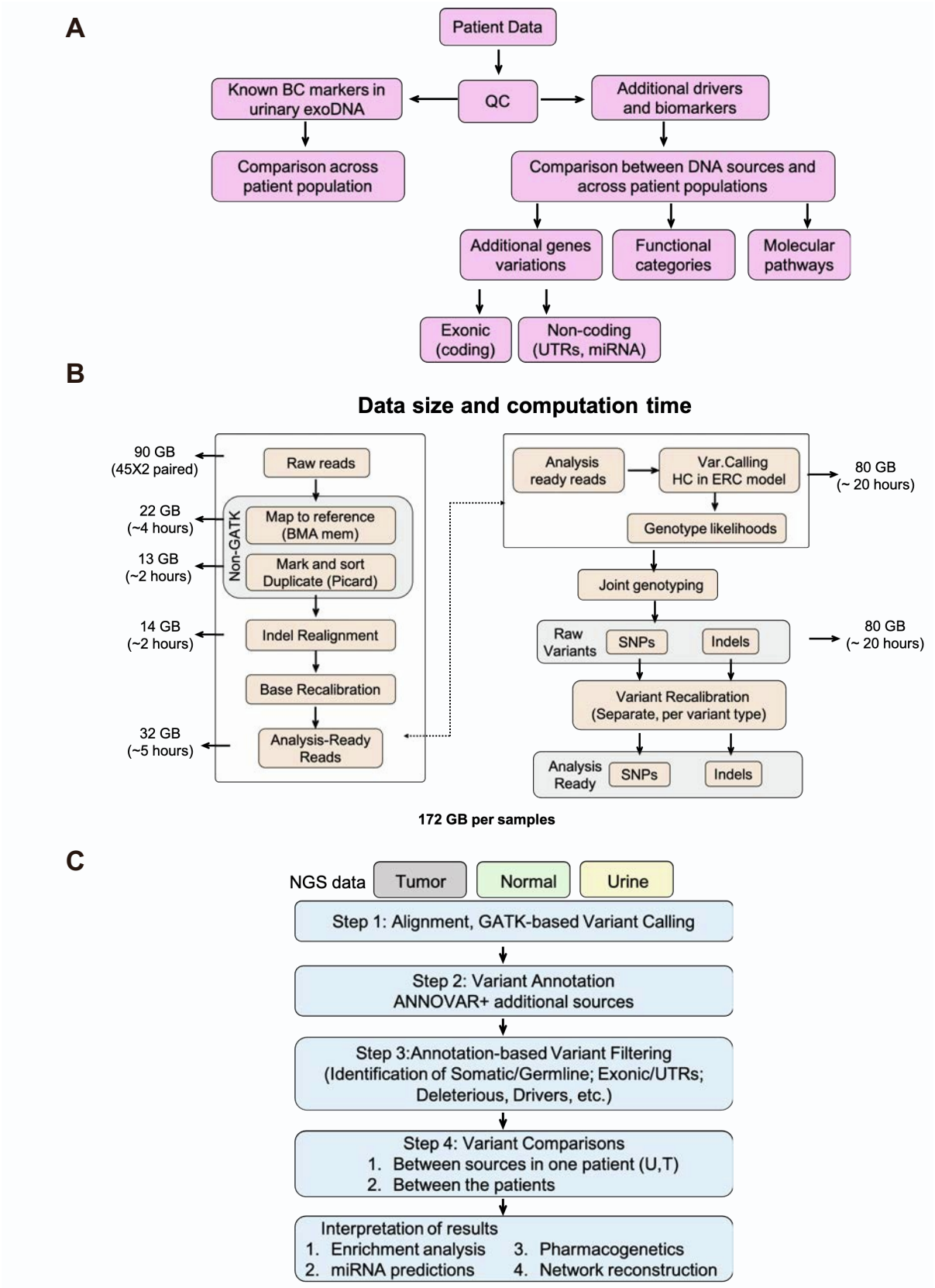
Supplementary Figure 3. Full-size autographs of the Western blots and Coomassie staining. (**A**) Lane 1: Healthy human urine exosome lysate. Lane 2: Healthy human serum exosome lysate. (**B**) Coomassie

blue staining of the fractions 7-11, 17-19 and UC exosome pellets. The presence of albumin was found in fractions 18 and 19. THP and albumin were present in the UC pellet. (**C**) Fraction 7-11 of urine exosome lysate. Molecular weights are indicated in writing under each autograph. (**D**) DNA was isolated from healthy sample 8 (H8) without DNase I (left) or with DNase I (right) to eliminate exogenous DNA and resultant DNA fragments were analyzed by capillary electrophoresis. (**E-F**) DNA was isolated from BC tumor biopsies (**E**), matched serum (**F**) from P2 and P5 and analyzed by capillary electrophoresis.

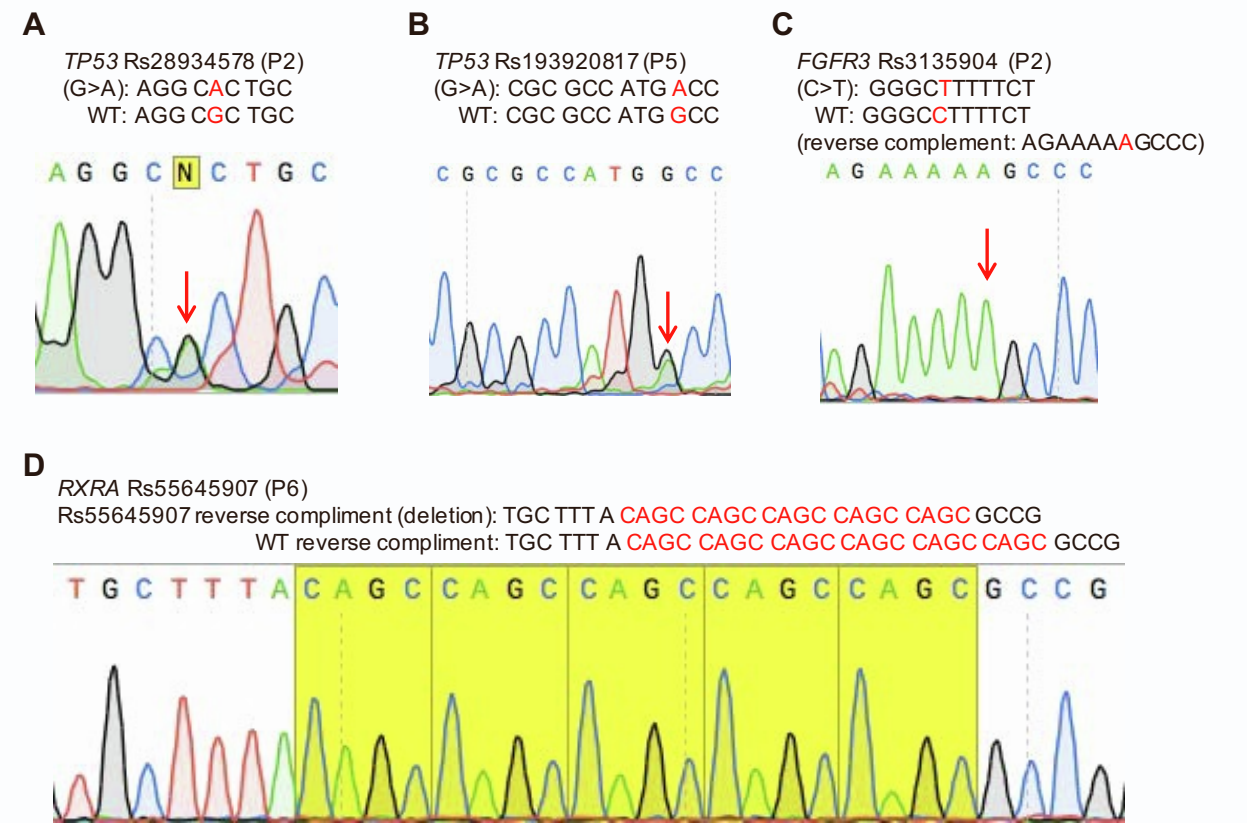


Supplementary Figure 4. PCR & Sanger sequencing of BC hotspots in patient samples. (**A**) Gel electrophoresis of PCR hotspot targets in urine exoDNA samples in six BC patients. (**B**) Representative

Sanger sequencing alignment of *TP53* hotspot PCR product in PBMC, tumor, and urine exoDNA from patient 2.


A
Patient Data
Known BC markers in urinary exoDNA
QC
Additional drivers and biomarkers
Comparison across patient population
Comparison between DNA sources and across patient populations
Additional genes variations
Functional categories
Molecular pathways
Exonic (coding)
Non-coding (UTRs, miRNA)
B
Data size and computation time
90 GB (45X2 paired)
Raw reads
22 GB (~4 hours)
Non-GATK
Map to reference (BMA mem)
13 GB (~2 hours)
Mark and sort Duplicate (Picard)
14 GB (~2 hours)
Indel Realignment
Base Recalibration
32 GB (~5 hours)
Analysis-Ready Reads
Analysis ready reads
Var.Calling HC in ERC model
80 GB (~ 20 hours)
Genotype likelihoods
Joint genotyping
Raw Variants
SNPs
Indels
80 GB (~ 20 hours)
Variant Recalibration (Separate, per variant type)
Analysis Ready
SNPs
Indels
172 GB per samples
C
NGS data
Tumor
Normal
Urine
Step 1: Alignment, GATK-based Variant Calling
Step 2: Variant Annotation
ANNOVAR+ additional sources
Step 3:Annotation-based Variant Filtering
(Identification of Somatic/Germline; Exonic/UTRs;
Deleterious, Drivers, etc.)
Step 4: Variant Comparisons
1. Between sources in one patient (U,T)
2. Between the patients
Interpretation of results
1. Enrichment analysis
2. miRNA predictions
3. Pharmacogenetics
4. Network reconstruction

Supplementary Figure 5. Bioinformatics analysis using NGS data for normal and tumor tissues, serum and urine exoDNA. (**A**) Analytic workflow; (**B**) High-throughput data analysis; (**C**) Downstream analysis.



Supplementary Figure 6. Sanger sequencing confirmed select variants in genes frequently mutated in bladder cancer. Tumor DNA isolated for the P2, P5 and P6 sample sets, which showed identical variants in the urine exoDNA and tumor tissue. DNA was amplified with primers flanking the identified variants and Sanger sequencing performed (for primers see Supplementary Table 2). (**A, B**) Confirmed variant for exonic variants in *TP53* sequence (P2 and P5, respectively). (**C**) Confirmed exonic variant in *FGFR3* sequence (P2). (**D**) Confirmed deletion in 3'UTR region in *RXRA* (P6).

Supplementary Tables

Supplementary Table 1. Bladder cancer patient information.

Information		Biopsy			Cystectomy		Treatment	
Patient ID	**Gender**	**Histology**	**Grade**	**Invasive**	**Positive Nodes (Y/N)**	**LVI (Y/N)**	**Neo-adjuvant Chemo**	**BCG/Intravesical Tx**
P1	Male	Papillary UCC	Low	Non-invasive	No	No	Ifos/Adria(2) DD-MVAC(4)	No
P2	Female	Squamous	High	Non-invasive	No	No	CGI-5 cycles	No
P3	Female	Papillary UCC	Low	Non-invasive	-	-	No	BCG/Mitomycin
P4	Male	UCC	High	Invasive	No	No	GEM/Cis 4 cycles	Yes (BCG)
P5	Male	UCC	High	Invasive	Yes	No	DD-MVAC 4 cycles	No
P6	Male	Small Cell/UCC	-	Invasive	No	No	Ifos/Adria(1) Etop/Cis (3)	No
P7	Male	UCC	High	invasive	Yes	-	Tax/Carbo, Gem/Cis, Pembro	No
P8	Male	Papillary	Low	Non-invasive	-	-	N/A	Yes (BCG)
P9	Male	Papillary	High	Non-invasive	-	-	N/A	Yes (BCG)
P10	Male	UCC, CIS	High	Non-invasive	No	No	No	Yes (BCG)

Supplementary Table 2. Primer sets for PCR amplification and Sanger sequencing.

Gene	Use	Forward – 5’	Reverse – 3’
FGFR3-1	PCR	CATGTCTTTGCAGCCGAGGA	GGCAGCTCAGAACCTGGTAT
FGFR3-2	PCR	GTGACCGAGGACAACGTGAT	TCGGTCAAACAAGGCCTCAG
FGFR3-3	PCR	CCCTGAGCGTCATCTGCC	ACCTTGCTGCCATTCACCTC
HRAS-1	PCR	GCGCCAGGCTCACCTCTAT	CTGGGCCTGGCTGAGCA
HRAS-2	PCR	ACTGGTGGATGTCCTCAAAAGA	AGAGGCTGGCTGTGTGAACT
KDM6A	PCR	ACACAACCAGCATTTACTTTTCCT	ATTGGCCAAAGGCTGCCC
TP53-1	PCR	GGCAACTGACCGTGCAAGT	TGCTGTCCCCGGACGATATT
TP53-2	PCR	AAGAAGCCCAGACGGAAACC	TCACCCATCTACAGTCCCCC
TP53-3	PCR	AACCCCTCCTCCCAGAGAC	CCAGGCCTCTGATTCCTCAC
TP53-4	PCR	TATGGAAGAAATCGGTAAGAGGTGG	ATCTTGGGCCTGTGTTATCTCC
TP53-5	PCR	CTGAGGCATAACTGCACCCT	TCCTTACTGCCTCTTGCTTCTC
TP53-6	PCR	GCTGCTCACCATCGCTATCT	TACTCCCCTGCCCTCAACAA
PIK3CA-1	PCR	CATCTGTGAATCCAGAGGGGAA	AGCACTTACCTGTGACTCCAT
PIK3CA-2	PCR	ACATTCGAAAGACCCTAGCCTT	AATCGGTCTTTGCCTGCTGA
TERT	PCR	AGTGGATTCGCGGGCACAGA	CAGCGCTGCCTGAAACTC
FGFR3-4	PCR	CCTGAAGATGGGAGCCTTTAC	CCTGGGACACACAGCAATTA
FGFR3-5	Sequencing	AGGCTGGACGTACATTCTTG	
RXRA	PCR	TGAGCCTCATACCTGTACCA	CTCTGTGGCATCTTCACTCC
RXRA	Sequencing	GGTGGCTAATGAGCTGATGTTA	
TP53-7	PCR	CATCACACCCTCAGCATCTC	GCCAGACCTAAGAGCAATCA
TP53-8	Sequencing	CATCACACCCTCAGCATCTC	

Supplementary Table 3. Total reads and coverage achieved by whole exome sequencing. Mapping rate (≥95%), Duplicate mapped reads (≤25%), Mean coverage (≥100X), Median coverage (≥50X) WES total reads & coverage. PBMC DNA was not available for patient 3 and this patient was excluded from further bioinformatics analysis. Raw sequencing metrics revealed a mean target coverage of 158-198X in PBMC samples, 138-162X in tumor samples, 31-334X in urine exosome samples, and 14-187X in serum exosome samples. Median target coverage ranged from 124-156X in PBMC samples, 103-127X in tumor samples, 1-138X in urine exosome samples, and 0-24X in serum samples. Median target coverage was likely reduced in urine and serum exosome samples due to whole-genome amplification being employed before library preparation, which is known to create bias in sequence fragment representation.

Type	Patient ID	Total reads x 10^6	Mapping rate (%)	Duplicate Mapped Reads (%)	Mean Coverage	Median Coverage	100X (%Targets)	75X (%Targets)	50X (%Targets)
PBMCs	1	166.57	99.78	12.75	196.35	155.77	68.03	73.68	77.9
PBMCs	2	162.04	99.8	13.23	198.36	156.26	68.36	73.86	77.78
PBMCs	4	150.11	99.8	11.66	186.92	150.57	67.18	73.29	77.63
PBMCs	5	142.86	99.76	13.26	168.41	132.65	63.18	71.1	76.72
PBMCs	6	138.96	99.7	13.2	158.76	124.98	61.02	70	76.31
Tumor	1	135.69	99.73	10.89	161.8	127.42	61.48	70.12	76.21
Tumor	2	131.81	99.73	11.3	157.93	118.28	57.96	67.85	75.18
Tumor	3	119.83	99.69	12.57	138.7	103.83	52.03	64.7	74.27
Tumor	4	126.69	99.73	11	153.08	114.17	56	65.81	73.64
Tumor	5	133.66	99.72	10.78	162.23	124.24	60.33	69.43	75.91
Tumor	6	128.38	99.73	13.91	149.83	109.06	54.27	65.52	74.28
Urine ExoDNA	1	313.38	99.28	18.11	334.59	138.64	39.59	47.82	58.56
Urine ExoDNA	2	247.14	98.38	24.03	233.48	63.12	23.67	29.86	39.25
Urine ExoDNA	3	238.27	96.4	42.73	162.47	6.86	8.88	10.95	14.43
Urine ExoDNA	4	239.51	78.11	76.43	31.29	1.14	1.58	2.08	2.98
Urine ExoDNA	5	249.37	98.75	32.85	199.52	20.47	11.62	14.94	20.64
Urine ExoDNA	6	260.87	98.9	36.09	185.7	26.2	9.64	13.4	20.28
Serum ExoDNA	1	163.31	87.06	75.18	13.99	0	0.6	0.77	1.08
Serum ExoDNA	2	255.72	97.39	39.18	187.52	23.95	24.08	29.04	36.44
Serum ExoDNA	3	259.5	94.78	47.39	141.96	3.93	12.94	15.42	19.33
Serum ExoDNA	4	264.42	95.95	65.09	148.16	0	5.97	6.93	8.46

Serum ExoDNA	5	255.88	93.14	47.93	122.54	21.31	20.08	25.14	32.88
Serum ExoDNA	6	256.7	89.06	78.58	33.8	0	1.29	1.57	2.12

Below quality threshold

Supplementary Table 4. Total variants, concordance and contamination analysis. (**A**) Total variants by sample, (**B**) Concordance, PBMCs (Normal, N) vs Tumor (T), (**C**) Concordance, PBMCs (Normal, N) vs Urine (U), (**D**) Concordance, PBMCs (Normal, N) vs Serum (S), (**E**) Contamination, PBMCs (Normal, N) vs Tumor (T), Urine (U) and Serum (S), (**F**) Summary of QU analysis.

A. Total variants by sample

	P1	P2	P4	P5	P6	Total Variants
PBMCs	1,055,918	954,048	879,234	908,288	948,607	
Serum	**28,197**	515,271	**140,414**	438,430	**34,241**	1,156,553
Tumor	944,394	934,908	1,053,203	874,486	872,261	4,679,252
Urine	1,214,430	851,080	**92,435**	524,199	637,053	3,319,197
Total variants	3,242,939	3,255,307	2,165,286	2,745,403	2,492,162	

B. Concordance, PBMCs (Normal, N) vs Tumor (T)

	P1N	P2N	P4N	P5N	P6N
P1T	**99.90%**	39.11%	40.33%	40.62%	39.06%
P2T	39.21%	**99.84%**	37.81%	39.78%	37.43%
P4T	40.31%	37.67%	**99.86%**	40.08%	38.07%
P5T	40.65%	39.78%	39.98%	**99.88%**	39.09%
P6T	38.88%	37.13%	38.12%	38.59%	**99.92%**

C. Concordance, PBMCs (Normal, N) vs Urine (U)

	P1N	P2N	P4N	P5N	P6N
P1U	**98.86%**	32.74%	34.62%	35.01%	31.30%
P2U	34.90%	**98.56%**	34.19%	36.00%	31.89%
P4U	44.85%	45.05%	***67.56%***	48.70%	43.95%
P5U	41.67%	39.62%	40.75%	**95.03%**	39.17%
P6U	35.83%	32.78%	34.25%	36.22%	**92.19%**

D. Concordance, PBMCs (Normal, N) vs Serum (S)

	P1N	P2N	P4N	P5N	P6N
P1S	***49.05%***	71.52%	42.95%	42.13%	38.98%
P2S	41.59%	**97.94%**	42.21%	42.08%	39.36%
P4S	55.02%	52.07%	***66.84%***	55.54%	52.81%
P5S	38.79%	38.42%	38.84%	**98.20%**	35.84%
P6S	51.79%	51.97%	50.82%	48.18%	***49.14%***

E. Contamination, PBMCs (Normal, N) vs Tumor (T), Urine (U) and Serum (S)

	N	T	U	S
P1	0.37%	0.51%	**1.38%**	***98.87%***
P2	0.36%	**0.70%**	**1.31%**	**2.35%**
P4	0.32%	0.33%	***94.87%***	***99.51%***
P5	0.55%	**0.75%**	**2.78%**	**1.55%**
P6	0.43%	**0.65%**	**4.48%**	***98.99%***

F. Summary of QU analysis. Conc: concordance; Cont: contamination.

	N		T		U		S	
	Conc	**Cont**	**Conc**	**Cont**	**Conc**	**Cont**	**Conc**	**Cont**
P1	OK	Possible	OK	Possible	OK	Possible	Possible swap	Massive
P2	OK	Possible	OK	Possible	Possible swap	Massive	Negative	Massive
P4	OK	OK	OK	OK	Possible swap	Massive	Possible swap	Massive
P5	OK	Possible	OK	Possible	OK	Possible	Negative	Massive
P6	OK	Possible	OK	Possible	OK	Possible	Possible swap	Massive

Supplementary Table 5. Read depth per individual somatic variants identified in the study. Normal: matched PBMCs.

Variation ID	Normal (PBMCs)					Tumor					Urine					Serum				
	P1	P2	P4	P5	P6	P1	P2	P4	P5	P6	P1	P2	P4	P5	P6	P1	P2	P4	P5	P6
rs10415095						4	3	11	7		27								10	
rs11343599						4	4		2		8			10					6	
rs58312807	4				4	3	5	3	5	4	4			10	11				17	
rs1130214						6	5		3		27						18			
rs2976396	6			8			5	6		5	193	2212		226	95					
rs13258775			4				5		6	3					3					
rs251860							3	6	2		3				5					
rs1051782	4						5		3	3										
rs2422978	5							4	6	6	3									
rs55645907	3			10		2	7	3		2	6	8			4		5			
rs1045570				5		3			4		9						10			
rs4842194	7		3	6		4	5		12		8	6								
rs34109509							3										25			
rs35280127	7						7				14									
rs3135904			3	8	3		2	9	3	3	37	20		18						
rs28934578							151													
rs193920817									128											
rs1800372		115					58					723	5				442			
rs9266												12			32					
rs712		8		4	6	7		3		6					3					
rs3828609									2											
rs1057016							2								2					
rs3173956	4				5			2			3									
rs704010					7	2	2		16		30			22	4					
rs10875943	10					7	3	3			17			6			158			
rs10248903	3						3		3		15	2			2					
rs7931342			5			3	7	3	4											
rs2981582	2						4	3			5	68								
rs5768709		2				4	3				8	3								
rs7832232	2						2		2		5	18								
rs1883924		4				3					7	5							5	
rs4939827		2					2					9			2				4	
rs4986938	125					111					557		5			8				
rs7504990					5						429	206		49	225					
rs4132601	3					5			4	3										
rs2367202					3	3	3				2									
rs3176336	2					2				11	6									
rs5030625	3					2			4			5								
rs9340799					5		2			3		4								
rs2234693					3					2		4			3					

rs1138272		117					60					844					187			
rs11611238		36					33					88					2			
rs17632542				266					264					198					751	
rs17634425				3					5					4					7	
rs1799939				302					291					589					11	
rs1801270		251					232					52					33			
rs2107425		5								3		17					61			
rs2479106		4					5					3					10			

Supplementary Table 6. Total shared & unique somatic variants in driver genes in tumor samples.

Shaded cells represent the number of variant unique for a specific patient. Clear cells show the overlap between the two patients.

	P1	P2	P4	P5	P6	Total
P1	90	6	11	9	5	124
P2	6	109	12	10	7	144
P4	11	12	71	11	6	117
P5	9	10	11	110	5	152
P6	5	7	6	5	81	107
	Overlap of variants between patients					
	Variants unique for a specific patient					

Supplementary Table 7. Number of variations predicted by GATK. GATK-based variant analysis using DNA isolated from tumor tissues, exoDNA from urine and serum, and matched normal (PBMC) DNA as the reference sequence.

Variation type	Normal (PBMCs)				
	P1	P2	P4	P5	P6
Exonic	23,292	23,647	22,878	22,966	24,052
Exonic; splicing	11	9	10	14	13
ncRNA_splicing	16	18	16	17	22
ncRNA_UTR3	239	214	209	222	250
ncRNA_UTR5	90	75	82	79	84
ncRNA_UTR5; ncRNA_UTR3	1	1	1	1	1
Splicing	146	134	137	148	151
UTR3	12,858	12,177	11,258	11,830	12,801
UTR5	4,870	4,801	4,492	4,675	4,685
UTR5;UTR3	11	12	8	11	12
Downstream	8,610	7,678	6,795	7,201	7,924
Intergenic	551,475	488,321	450,182	462,446	474,249
Intronic	401,210	367,773	337,914	351,450	375,700
ncRNA_exonic	5,476	5,321	4,928	5,228	5,236
ncRNA_intronic	34,464	31,578	28,947	30,253	31,093
Upstream	12,666	11,777	10,960	11,305	11,916
Upstream; downstream	483	512	417	442	418

Variation type	Tumor				
	P1	P2	P4	P5	P6
Exonic	23,252	23,792	22,765	22,953	23,097
Exonic; splicing	11	10	10	14	13
ncRNA_splicing	20	14	17	20	21
ncRNA_UTR3	217	212	220	226	233
ncRNA_UTR5	87	76	73	78	85
ncRNA_UTR5; ncRNA_UTR3	1	1	1	1	0
Splicing	140	141	131	143	152
UTR3	11,895	12,001	11,498	11,969	11,480
UTR5	4,646	4,666	4,432	4,539	4,438
UTR5; UTR3	10	11	7	10	9
Downstream	7,476	7,326	8,141	7,105	7,133
Intergenic	487,245	478,321	556,140	436,611	437,281
Intronic	361,076	360,185	399,713	345,189	342,511
ncRNA_exonic	5,296	5,199	4,997	5,089	4,984
ncRNA_intronic	31,316	31,262	33,656	29,313	29,470
Upstream	11,290	11,180	10,977	10,787	10,934
Upstream; downstream	416	511	425	439	420

Variation type	Urine				
	P1	P2	P4	P5	P6
Exonic	23,689	24,205	9,773	22,064	23,359
Exonic; splicing	10	9	5	9	13
ncRNA_splicing	25	25	6	18	19
ncRNA_UTR3	250	229	41	147	143
ncRNA_UTR5	85	69	13	48	54
ncRNA_UTR5; ncRNA_UTR3	1	8	0	0	1
Splicing	168	183	87	193	240
UTR3	13,970	11,370	1,587	7,705	7,973
UTR5	4,684	4,008	642	2,726	2,610
UTR5;UTR3	10	11	2	7	11
Downstream	11,068	7,868	672	4,876	4,728
Intergenic	635,479	417,525	39,441	252,902	318,299
Intronic	465,532	343,203	35,790	206,794	250,256
ncRNA_exonic	6,013	4,905	755	3,656	3,308
ncRNA_intronic	39,214	26,926	2,612	16,571	20,251
Upstream	13,688	10,054	959	6,144	5,572
Upstream; downstream	544	482	50	339	216

Variation type	Serum				
	P1	P2	P4	P5	P6
Exonic	4,609	23,779	7,080	25,240	3,758
Exonic; splicing	4	9	6	13	3
ncRNA_splicing	0	15	2	12	3
ncRNA_UTR3	15	171	48	140	7
ncRNA_UTR5	3	48	5	35	1
ncRNA_UTR5; ncRNA_UTR3	0	0	0	0	0
Splicing	30	235	81	281	52
UTR3	656	8,523	1,961	7,980	710
UTR5	306	3,164	832	3,098	319
UTR5; UTR3	0	7	1	6	0
Downstream	122	4,431	1,170	3,627	243
Intergenic	10,336	239,596	69,886	189,446	14,448
Intronic	10,740	208,731	51,924	185,453	12,924
ncRNA_exonic	303	3,278	1,127	3,097	294
ncRNA_intronic	771	16,402	4,660	13,690	955
Upstream	288	6,570	1,568	5,985	495
Upstream; downstream	14	312	63	327	29

Supplementary Table 8. Antibodies used in this study.

Primary antibodies							
Antigen	Specificity	Host	Vendor	Catalog No	Lot No	Used in	Dilution
CD9	Human, Mouse, Rat	Rabbit	Abcam	Ab92726	GR237847-20	TEM, WB	1:300, 1:1000
Flotillin-1	Human	Rabbit	Santa Cruz	sc25506	H1914	WB	1:300
Secondary antibodies							
Ab Type	Specificity	Host	Conjugate	Vendor	Catalog No	Used in	Dilution
IgG	Rabbit	Goat	HRP	Sigma	A0545	WB	1:2000