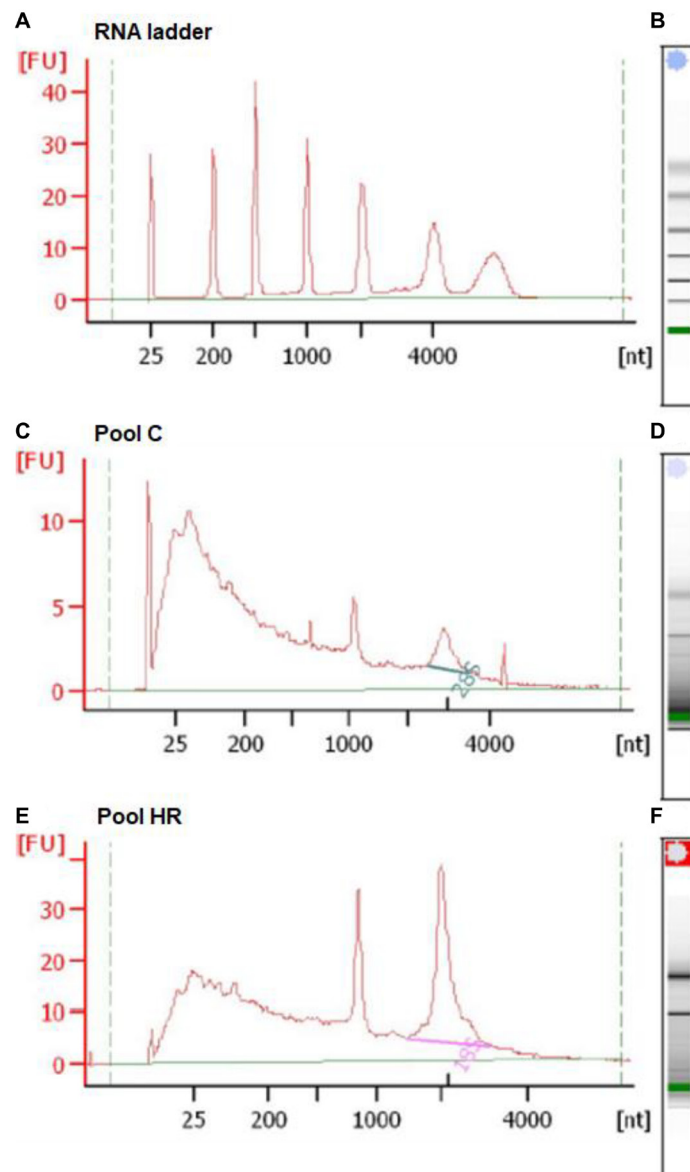


## Transcriptome analyses of urine RNA reveal tumor markers for human bladder cancer: validated amplicons for RT-qPCR-based detection

### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1: Analysis of RNA pools reveals high RNA degradation.** The Agilent RNA 6000 Pico Kit was used to analyze the integrity of the RNA in pools C and HR. (A, C and E) show the electropherograms of a control RNA ladder, pool C and pool HR, respectively. (B, D and F) show the gel images of a control RNA ladder, pool C and pool HR respectively. The absence of distinct peaks (C and E) and bands (D and F) in comparison to the control RNA ladder indicate significant RNA degradation.

**Supplementary Table 1: Clinical characteristics of patients**

Pool	Patient Number	Sex	Grade	Risk	Stage	Recurrence
<b>C</b>	48	f	-	-	-	-
	50	m	-	-	-	+
	80	m	-	-	-	+
	138	m	-	-	-	+
	160	m	-	-	-	-
	166	f	-	-	-	+
	170	m	-	-	-	-
<b>HR</b>	41	m	G3, HG	HR	Ta	-
	46	m	G2, HG	HR	Ta	-
	107	f	G2, HG	HR	Ta	+
	128	m	G3, HG	HR	Ta	-
	159	m	G3, HG	HR	T2-4	-
	164	m	G3, HG	HR	Ta	+
	183	m	HG	HR	T2-4	-

Description: f = female, m = male, C = control group, HR = high risk, HG = high grade, Ta = noninvasive papillary BCa, T2-4 = invasive BCa, G2/G3 = grading of BCa.

**Supplementary Table 2: Final cDNA pool composition for sequencing**

Library #	Content	Concentration [ng/μl]	Contribution in final pool [%]
<b>1</b>	C	24.4	21
<b>2</b>	C	16.5	32
<b>3</b>	HR	19.8	26
<b>4</b>	HR	23.6	21

Shown are content, concentration and contribution in final pool for cDNA libraries 1–4.

**Supplementary Table 3: Classification of up- and down-regulated RNAs in pooled samples of the control group C and the HR group**

<b>(A) Up-regulated coding transcripts in urine of high risk patients compared to healthy subjects</b>							
<b>Transcript ID</b>	<b>Name</b>	<b>length [kb]</b>	<b>HR [TPM]</b>	<b>C [TPM]</b>	<b>log(HR/C)</b>	<b>TSL</b>	<b>Biotype</b>
ENST00000525608	GLYATL1-206	0.573	37.616	0.895	1.624	2	Pc
ENST00000320868	HBA1-201	0.577	163.828	8.190	1.301	1	Pc
ENST00000472694	HBA1-203	0.674	42.293	2.529	1.223	1	Ri
ENST00000487791	HBA1-204	0.410	12.091	1.361	0.948	1	Ri
ENST00000397797	HBA1-202	0.504	58.325	2.586	1.353	2	Pc
ENST00000397806	HBA2-201	0.513	44.780	2.261	1.297	2	Pc
ENST00000482565	HBA2-203	0.640	24.005	2.314	1.016	1	Pc
ENST00000484216	HBA2-204	0.403	12.415	2.224	0.747	1	Pc
ENST00000335295	HBB-201	0.628	128.769	7.801	1.218	1	Pc
ENST00000485743	HBB-204	0.680	24.824	7.685	0.509	1	Pc
ENST00000475226	HBB-203	0.319	29.203	1.980	1.169	2	Ri
ENST00000633227	HBB-205	0.609	94.092	5.025	1.272	3	NMD
ENST00000307407	CXCL8-201	1.705	618.357	148.984	0.618	1	Ri
ENST00000401931	CXCL8-202	0.700	365.908	86.693	0.625	1	Pc
ENST00000483500	CXCL8-203	0.599	162.685	45.813	0.550	2	Pc
ENST00000466565	MBNL1-214	1.096	25.787	2.723	0.976	1	Pc
ENST00000496817	S100A6-205	0.673	79.051	19.216	0.614	2	Pc
ENST00000368720	S100A6-202	0.673	70.632	17.232	0.613	3	Pc
ENST00000368719	S100A6-201	0.665	120.508	28.035	0.633	1	Pc
ENST00000462776	S100A6-203	0.479	13.011	4.239	0.487	2	Pc
ENST00000422514	RPL23A-204	0.970	127.601	38.520	0.520	1	Pc
ENST00000496182	RPL23A-206	0.636	35.841	11.507	0.493	3	Pc
ENST00000355731	RPL23A-201	0.587	36.773	11.417	0.508	5	Pc
ENST00000394935	RPL23A-202	0.580	36.805	10.828	0.531	5	Pc
ENST00000582736	RPL23A-209	0.595	151.180	49.356	0.486	1	Ri
ENST00000368567	RPS27-201	0.359	779.327	258.917	0.479	1	Pc
ENST00000493224	RPS27-203	0.573	44.177	14.822	0.474	2	Ri
<b>(B) Down-regulated coding transcripts in urine of high risk patients compared to healthy subjects</b>							
<b>Transcript ID</b>	<b>Name</b>	<b>length [kb]</b>	<b>HR [TPM]</b>	<b>C [TPM]</b>	<b>log(HR/C)</b>	<b>TSL</b>	<b>Biotype</b>
ENST00000585467	ZNF382-208	0.550	17.540	293.821	-1.224	4	Pt
ENST00000637522	RYS3-227	0.137	0.236	3.701	-1.196	5	Pt
ENST00000390499	TRAJ38-201	0.062	0.520	6.923	-1.124	NA	TR J gene
ENST00000520700	TRAM1-203	0.542	1.630	20.115	-1.091	5	Pt
<b>(C) Up-regulated noncoding transcripts in urine of high risk patients compared to healthy subjects</b>							
<b>Transcript ID</b>	<b>Name</b>	<b>length [kb]</b>	<b>HR [TPM]</b>	<b>C [TPM]</b>	<b>log(HR/C)</b>	<b>TSL</b>	<b>Biotype</b>
ENST00000414790	H19-203	2.348	1.47	7.97	0.73	1	LncRNA
ENST00000412788	H19-202	2.281	1.24	7.36	0.77	1	LncRNA
ENST00000439725	H19-209	1.929	0.90	6.42	0.85	2	LncRNA
ENST00000411861	H19-201	1.771	1.30	5.35	0.61	2	Ri
ENST00000417089	H19-204	1.090	0.69	5.56	0.90	5	LncRNA
ENST00000431095	H19-207	0.944	0.87	6.31	0.86	5	Ri

ENST00000428066	H19-206	0.989	1.60	7.99	0.70	3	LncRNA
ENST00000436715	H19-208	0.861	1.29	4.76	0.57	5	Ri
ENST00000447298	H19-211	0.592	1.32	10.63	0.90	2	LncRNA
ENST00000442037	H19-210	0.798	0.80	3.49	0.64	3	LncRNA
ENST00000607600	AL136131.3-201	0.188	463.57	100.38	0.66	NA	LncRNA
ENST00000636484	RN7SK-201	0.328	1241.05	389.88	0.50	NA	SnRNA

**(D) Down-regulated noncoding transcripts in urine of high risk patients compared to healthy subjects**

Transcript ID	Name	length [kb]	HR [TPM]	C [TPM]	log(HR/C)	TSL	Biotype
ENST00000625038	AL008636.1-201	1.018	387.21	596.22	-0.19	NA	LncRNA
ENST00000521218	AC103957.2-201	0.510	588.68	740.57	-0.10	2	LncRNA
ENST00000523087	HDAC2-AS2-217	0.493	2299.64	3,452.42	-0.18	3	LncRNA
ENST00000451415	HDAC2-AS2-205	0.559	1982.01	3,032.37	-0.18	3	LncRNA
ENST00000387365	MT-TI-201	0.069	648.37	954.01	-0.17	NA	Mt tRNA
ENST00000387382	MT-TW-201	0.068	1008.28	1,383.40	-0.14	NA	Mt tRNA
ENST00000427161	AL135790.1-201	0.157	8300.05	11,224.37	-0.13	3	LncRNA
ENST00000562900	AC009035.1-201	0.717	1609.17	2,115.62	-0.12	3	LncRNA
ENST00000521908	AC131254.2-201	0.550	961.94	1,247.84	-0.11	3	LncRNA
ENST00000557618	AL049874.3-201	0.868	640.98	821.81	-0.11	3	LncRNA
ENST00000597502	AL158835.1-205	0.818	616.97	821.77	-0.12	5	LncRNA
ENST00000611240	AL158835.1-206	0.632	528.52	676.35	-0.11	5	LncRNA
ENST00000606512	AC036214.2-201	0.917	501.33	640.63	-0.11	NA	LncRNA
ENST00000557465	AL356022.1-201	0.483	456.93	590.92	-0.11	5	LncRNA

**(E) Up-regulated circular transcripts in urine of high risk patients compared to healthy subjects**

Transcript ID	Name	length [kb]	HR [TPM]	C [TPM]	log(HR/C)
hsa_circ_0037140	HBA1	0.444	1090.27	66.05	1.22
hsa_circ_0037139	HBA2	0.239	221.73	16.62	1.13
hsa_circ_0037141	HBA1	0.239	114.55	11.04	1.02
hsa_circ_0113405	SLC2A1	0.232	142.13	20.91	0.83
hsa_circ_0050104	GDF15	1.200	109.04	27.23	0.60
hsa_circ_0076608	VEGFA	1.430	303.62	77.71	0.59
hsa_circ_0014225	S100A6	0.390	391.17	102.25	0.58
hsa_circ_0014226	S100A6	0.683	314.28	87.06	0.56
hsa_circ_0014224	S100A6	0.231	105.43	29.96	0.55

**(F) Down-regulated circular transcripts in urine of high risk patients compared to healthy subjects**

Transcript ID	Name	length [kb]	HR [TPM]	C [TPM]	log(HR/C)
hsa_circ_0084758	TRAM1	0.176	0.66	41.33	-1.80
hsa_circ_0084756	TRAM1	0.383	0.81	21.56	-1.43
hsa_circ_0084757	TRAM1	0.447	0.98	18.51	-1.28
hsa_circ_0137075	TRAM1	0.380	1.15	16.15	-1.15
hsa_circ_0064279	SEC13	0.405	14.24	140.33	-0.99
hsa_circ_0065026	KIF15	0.472	3.19	26.96	-0.93
hsa_circ_0064275	SEC13	0.591	12.43	102.34	-0.92
hsa_circ_0109513	ZNF382	5.901	18.56	147.16	-0.90
hsa_circ_0084759	TRAM1	0.422	3.08	20.04	-0.81

Legend: Protein coding (Pc), Retained Intron (Ri), Processed transcript (Pt), Nonsense mediated decay (NMD), TSL not analyzed (NA), long noncoding RNA (LncRNA), Small nuclear RNA (SnRNA), Mitochondrial transfer RNA (Mt tRNA).

**Supplementary Table 4: Primer pairs and length of PCR amplicons**

Transcript	1. Forward Primer 5'→3' 2. Reverse Primer 5'→3' 3. TaqMan® probe1 5'→3'	Amplicon length (bp)
BIRC5	1. GACCACCGCATCTCTACATTCA 2. CAAGTCTGGCTCGTTCTCAGT 3. –	117
KRT20	1. AGCTGCGAAGTCAGATTAAGGA 2. GAAGTCCTCAGCAGCCAGTT 3. –	89
LASP1	1. CTCGGAACCATGAACCCCA 2. ATGCCAGAACTTATCCAGACAGT 3. –	87
OP18	1. AAAGACGCAAGTCCCATGAAG 2. AGCTTCCATTTTGTGGGTCAG 3. 5'-FAM-GCAGCTGGCTGAGAAACGAGAGCA-BHQ1-3'	146
UPK1A	1. CGGAAGGCTGACGTGAAGT 2. CGTCATGATTGAGCAAGAATGC 3. FAM-TGGCACATCTGGTCCCATGGA-BHQ-1	72
18S rRNA	1. CACATCCAAGGAAGGCAGCAG 2. GACTTGCCCTCCAATGGATCC 3. –	152
CXCL8	1. ACCGGAAGGAACCATCTCAC 2. GGCAAACTGCACCTTCACAC 3. –	108
RPS27	1. CGAGAACATGCCTCTCGCA 2. GCACCAGGCGTTTCTTCTTG 3. –	83
RPL23A	1. TGCCAACAAAATTGGGATCATCT 2. CCCAGCCCAACCAGAAATTG 3. –	115
GLYATL	1. GAGCATTCGAGAGCACTCCT 2. TGGGAGTTTCACTTCAAATTCATC 3. –	127
ZNF382	1. GAGGAGTGGCAGCAACTAGA 2. ATGTGAAACCCACAGATACGA 3. –	95
RYR3	1. GTGGGGCTATTCCAGTTGCT 2. AAATTGGGTTGGGTCAGGCA 3. –	147
TRAM1 (convergent)	1. ATCTGGTCAGCTTAGTGCGT 2. TGGGGATAAGCCCTCCATAAGA 3. –	111
S100A6 (convergent)	1. ACTTGGACCGGAACAAGGAC 2. AGGGTGTCTCCATCTTCCCT 3. –	126
TRAM1 (divergent)	1. GTTTGGGGCACATTCACTCTCATC 2. GGAGAAGTGCATTGCGCTGT 3. –	113, 320, 363, 384
S100A6 (divergent)	1. GGGTCCTCTCTGAGTCAAATCCA 2. CTGCCGGAGTACTTGTGGAAGA 3. –	150, 443

<sup>1</sup>FAM = 6-Carboxyfluorescein; BHQ = Black Hole Quencher™.