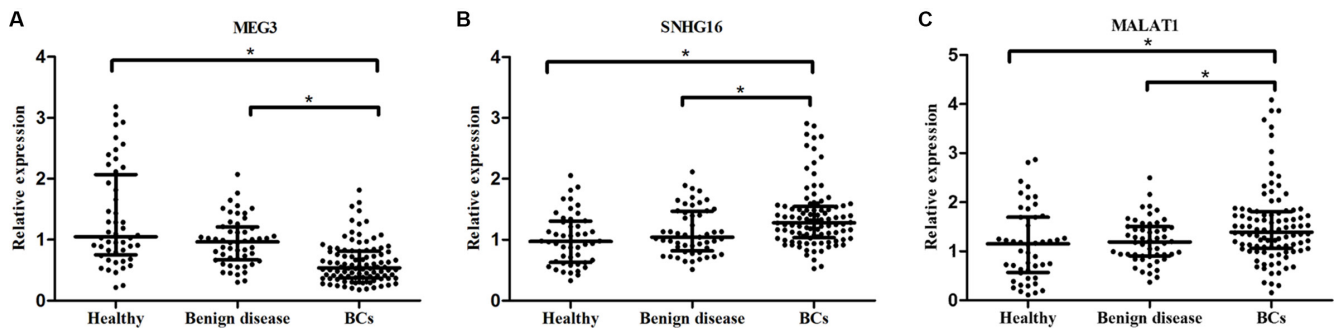
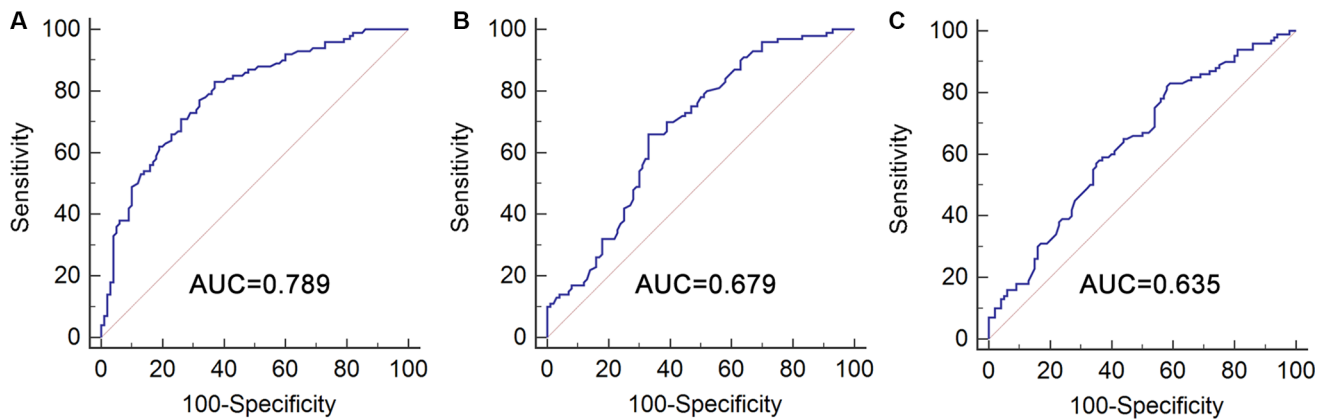


## Identification of a serum circulating lncRNA panel for the diagnosis and recurrence prediction of bladder cancer

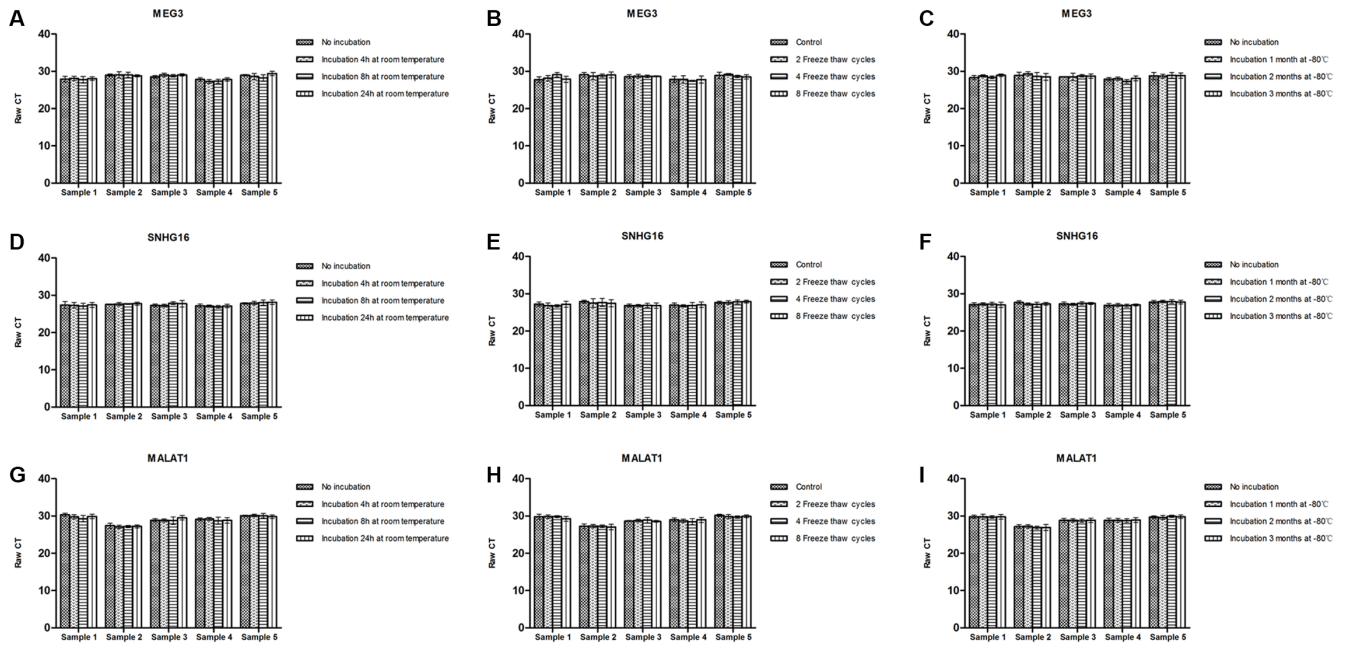
### Supplementary Materials



**Supplementary Figure S1: Validation of MEG3, SNHG16 and MALAT1 as biomarkers for detecting BC.** Expression levels of three selected lncRNAs (MEG3, SNHG16 and MALAT1) in serum samples in Healthy vs. Benign disease ( $p > 0.05$ ), Healthy vs. BCs ( $p < 0.01$ ) and Benign disease vs. BCs comparisons ( $p < 0.01$ ) in validation set.



**Supplementary Figure S2: Validation of diagnostic performance of selected lncRNAs for BC patients versus controls.** Receiver operating curves indicated the ability of the serum levels of three individual lncRNAs including MEG3 (A), SNHG16 (B) and MALAT1 (C) to differentiate BC patients from controls in validation set.



**Supplementary Figure S3: Stability of serum MEG3, SNHG16 and MALAT1.** MEG3, SNHG16, and MALAT1 serum levels remained stable when treated with prolonged exposure to room temperature, or multiple freeze-thaw cycles, or prolonged  $-80^{\circ}\text{C}$  incubation time.

**Supplementary Table S1: Characteristics of study participants in training set and validation set [median (interquartile range)]**

Variable	Training set	Validation set	<i>P</i> -Value
	<i>n</i> = 240	<i>n</i> = 200	
<b>Control (number)</b>	120	100	
<b>Age (years)</b>			0.33
≤ 60	46 (38.33%)	32 (32%)	
> 60	74 (61.67%)	68 (68%)	
<b>Sex</b>			0.71
Male	96 (80%)	82 (82%)	
Female	24 (20%)	18 (18%)	
<b>Healthy (number)</b>	52 (43.33%)	48 (48%)	
<b>Benign disease</b>			0.73
BPH	32 (26.67%)	21 (21%)	
Urolithiasis	16 (13.33%)	15 (15%)	
Cystitis	20 (16.67%)	16 (16%)	
<b>BC (number)</b>	120	100	
<b>Age (years)</b>			0.34
≤ 60	42 (35%)	29 (29%)	
> 60	78 (65%)	71 (71%)	
<b>Sex</b>			0.30
Male	97 (80.83%)	75 (75%)	
Female	23 (19.17%)	25 (25%)	
<b>Tumor stage</b>			0.63
Ta–T1	77 (64.17%)	61 (61%)	
T2–T4	43 (35.83%)	39 (39%)	
<b>Tumor grade</b>			0.59
Low grade	68 (56.67%)	53 (53%)	
High grade	52 (43.33%)	47 (47%)	
<b>Lymph node matastasis</b>			0.55
Negative	101 (84.17%)	87 (87%)	
Positive	19 (15.83%)	13 (13%)	

Abbreviations: BPH, benign prostatic hyperplasia; BC, bladder cancer.

**Supplementary Table S2: The selected lncRNA concentrations in BC tissues as compared with matched adjacent normal tissues [median (interquartile range)]**

<b>lncRNA</b>	<b>Controls</b>	<b>BCs</b>	<b>P</b>	<b>lncRNA</b>	<b>Controls</b>	<b>BCs</b>	<b>P</b>
MEG3	1.09 (0.53–2.08)	0.78 (0.50–1.27)	< 0.01	SPRY-IT1	0.97 (0.59–1.64)	3.10 (1.26–5.78)	< 0.01
SNHG16	1.26 (0.57–1.80)	3.10 (2.34–3.98)	< 0.01	TUG1	1.02 (0.56–2.15)	5.28 (3.64–7.23)	< 0.01
MALAT1	1.32 (0.54–2.17)	1.83 (0.81–3.21)	< 0.01	UCA1	1.10 (0.67–1.95)	2.37 (1.40–4.54)	< 0.01
PCAT-1	1.23 (0.44–2.08)	2.64 (1.47–5.52)	< 0.01	lincRNA-PRss16	0.99 (0.57–1.70)	1.32 (1.03–1.88)	< 0.01
GHET	1.25 (0.73–1.72)	1.55 (1.12–2.87)	< 0.01	GAS5	1.07 (0.65–1.60)	1.01 (0.52–1.76)	0.53
H19	1.13 (0.48–2.11)	2.32 (1.52–3.83)	< 0.01	BC039493	1.00 (0.71–1.41)	1.17 (0.76–1.59)	0.16
UBC1	1.07 (0.56–1.76)	3.92 (2.54–6.24)	< 0.01				

**Supplementary Table S3: Correlation between serum lncRNA concentrations and clinicopathological characteristics of patients with BC in validation set [median (interquartile range)]**

<b>Parameters</b>	<b>Total cases</b>	<b>MEG3</b>	<b>P</b>	<b>SNHG16</b>	<b>P</b>	<b>MALAT1</b>	<b>P</b>
<b>Age</b>			0.84		0.85		0.48
≤ 66	52	0.54 (0.37–0.79)		1.28 (0.97–1.53)		1.44 (1.09–1.86)	
> 66	48	0.54 (0.37–0.82)		1.28 (1.06–1.58)		1.35 (1.00–1.73)	
<b>Sex</b>			0.43		0.97		0.36
Male	75	0.57 (0.38–0.82)		1.31 (1.03–1.50)		1.36 (1.01–1.74)	
Female	25	0.51 (0.36–0.72)		1.25 (1.04–1.58)		1.49 (1.16–1.85)	
<b>Tumor stage</b>			0.03		0.50		0.72
Ta–T1	61	0.60 (0.41–0.83)		1.25 (1.05–1.49)		1.43 (1.09–1.76)	
T2–T4	39	0.51 (0.31–0.69)		1.33 (1.02–1.57)		1.36 (1.05–1.87)	
<b>Tumor grade</b>			0.73		0.06		0.71
Low grade	53	0.50 (0.37–0.82)		1.17 (0.98–1.49)		1.40 (1.00–1.80)	
High grade	47	0.57 (0.36–0.76)		1.37 (1.20–1.57)		1.38 (1.09–1.87)	
<b>Lymph node matastasis</b>			0.14		0.42		0.52
Negative	87	0.55 (0.40–0.83)		1.28 (1.02–1.54)		1.43 (1.09–1.78)	
Positive	13	0.48 (0.29–0.71)		1.33 (1.17–1.62)		1.10 (0.99–2.10)	

**Supplementary Table S4: Univariate Cox proportional hazards regression model analysis of recurrence-free survival in MIBC patients in validation set**

Parameters	Categories	Univariate analysis	
		HR (95% CI)	<i>p</i> Value
MEG3	Low vs. High	0.682 (0.305–1.525)	0.352
SNHG16	Low vs. High	1.526 (0.683–3.411)	0.303
MALAT1	Low vs. High	1.833 (0.794–4.228)	0.156

Abbreviations: HR, hazard ratio; CI, confidence interval.