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Supplemental information

CDKN2A copy number alteration in bladder cancer: Integrative analysis in patient-derived xenografts and cancer patients

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Figure S1

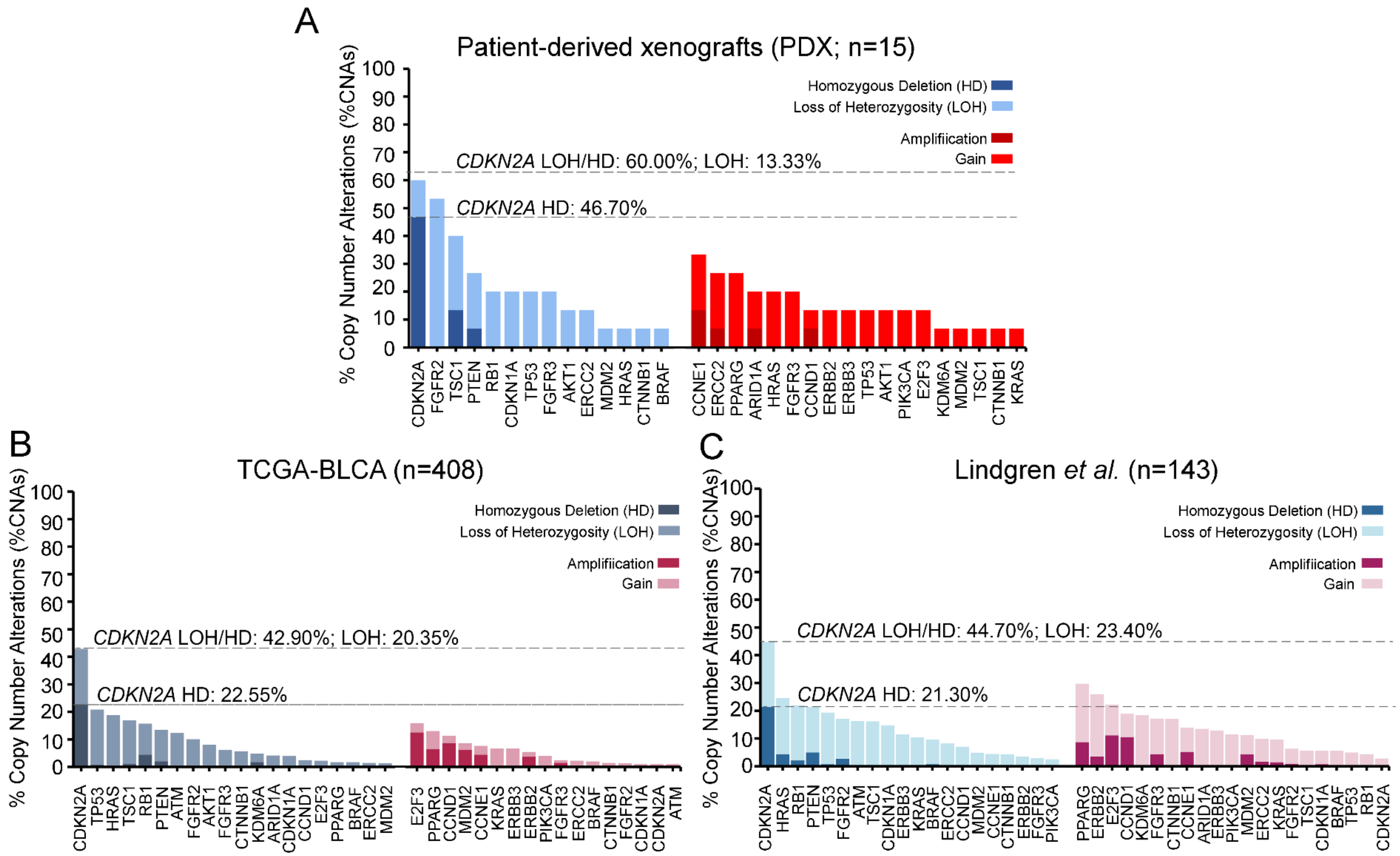

Figure S1. Bar graphs of gene level CNAs frequency (%) in PDX tumors samples (A), TCGA-BLCA dataset (B) and Lindgren *et al.* dataset (C).

Figure S2

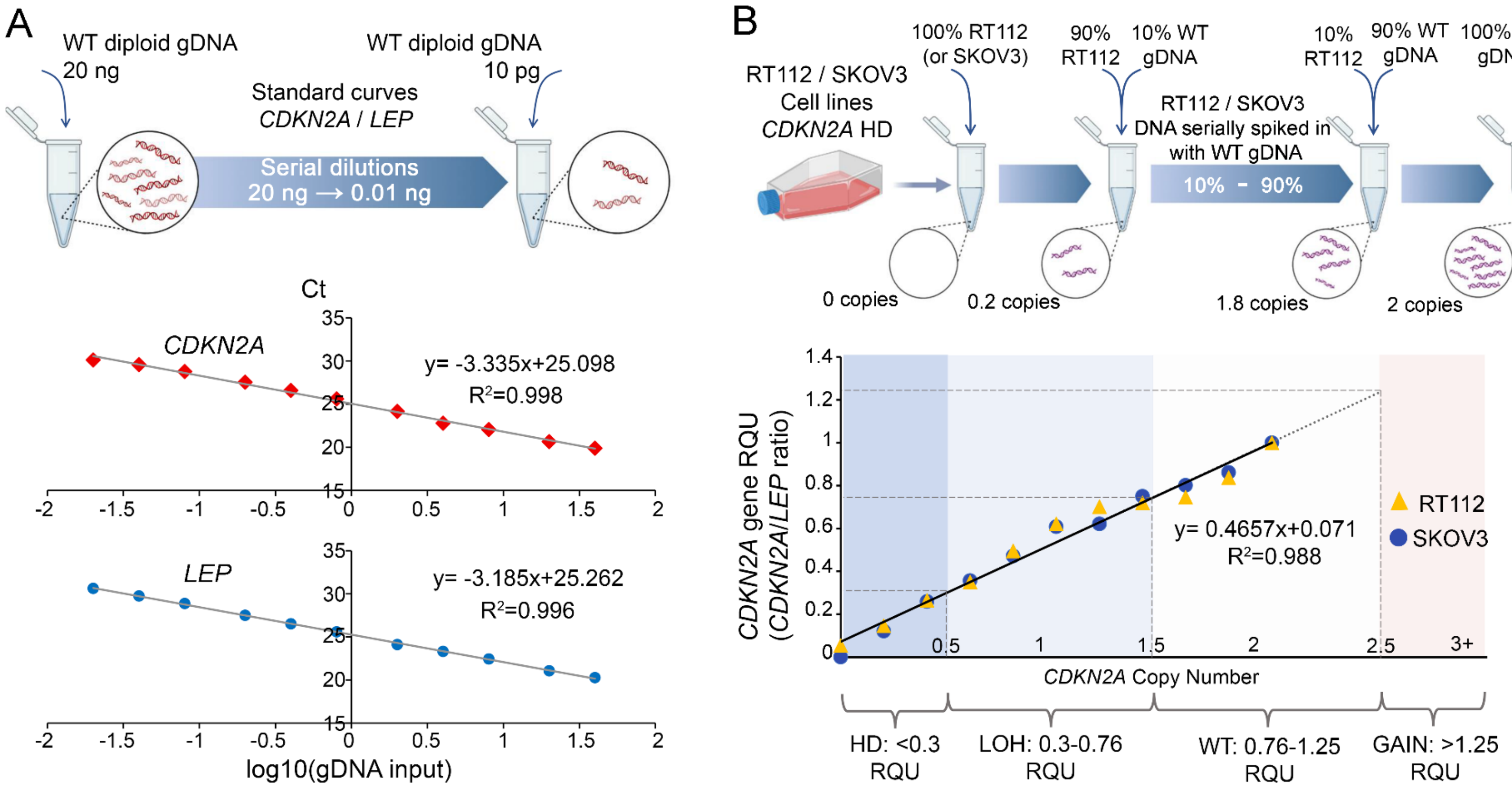

Figure S2. Development and validation of *in-house* qPCR-based method for *CDKN2A* copy-number status analysis. A. Standard curves of *CDKN2A* and *LEP* qPCR assays (range of gDNA: 0.01-20 ng). Both assays demonstrated high linearity, ensuring that PCR amplification efficiency (*CDKN2A*: E=99.9%; LEP: E=103.1%), of each target is constant over a broad concentration range. B. Standard curve for defining the optimal cut-offs for *CDKN2A* copy-number status. RT112 and SK-OV-3 cell lines, with known HD of *CDKN2A* (NCI-60/UBC-40 cell line panels) were spiked in with serially increasing amounts of *CDKN2A* diploid (wt) gDNA (0-100%). *CDKN2A* HD: *CDKN2A/LEP* ratio ≤0.30, LOH: *CDKN2A/LEP* ratio=0.30-0.76, diploid (wt): *CDKN2A/LEP* ratio =0.76-1.25, gain: *CDKN2A/LEP* ratio ≥1.25 RQU. gDNA: genomic DNA, HD: Homozygous deletion, LOH: Loss-of-heterozygosity, WT: Wild type, RQU: Relative quantitation units.

Figure S3

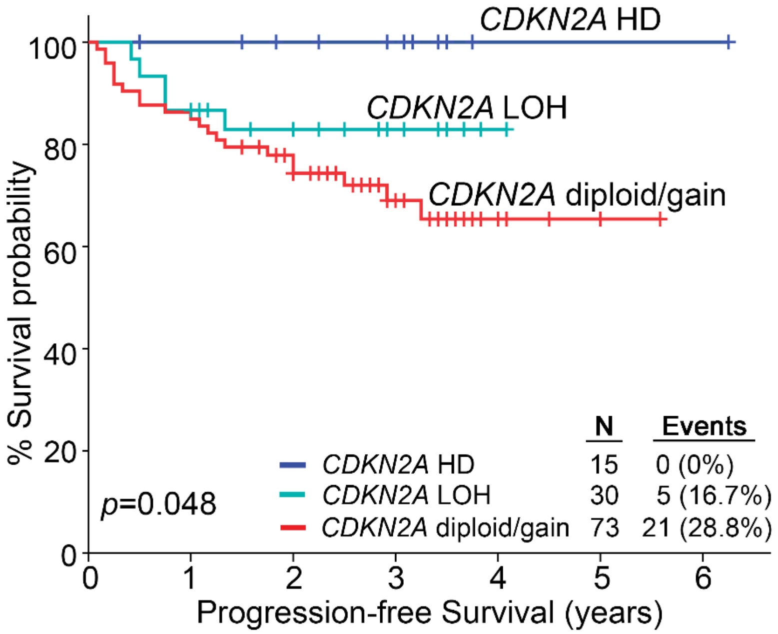

Figure S3. Kaplan-Meier survival curves for progression-free survival (PFS) of NMIBC patients according to HD *vs.* LOH *vs.* diploid/gain *CDKN2A* copy-number status. *p*-values calculated by log-rank test. HD: Homozygous deletion, LOH: Loss-of-heterozygosity

Figure S4

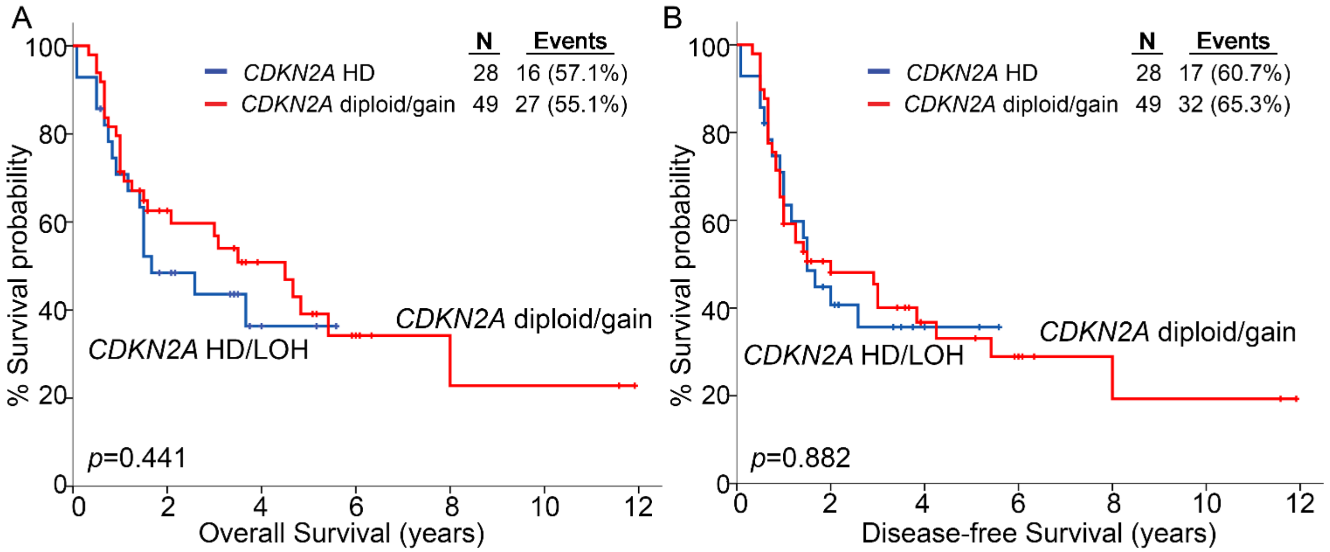

Figure S4. Kaplan-Meier survival curves for overall survival (OS) and Disease-free survival (DFS) of MIBC patients. *p*-values calculated by log-rank test. HD: Homozygous deletion; LOH: Loss-of-heterozygosity.

Figure S5

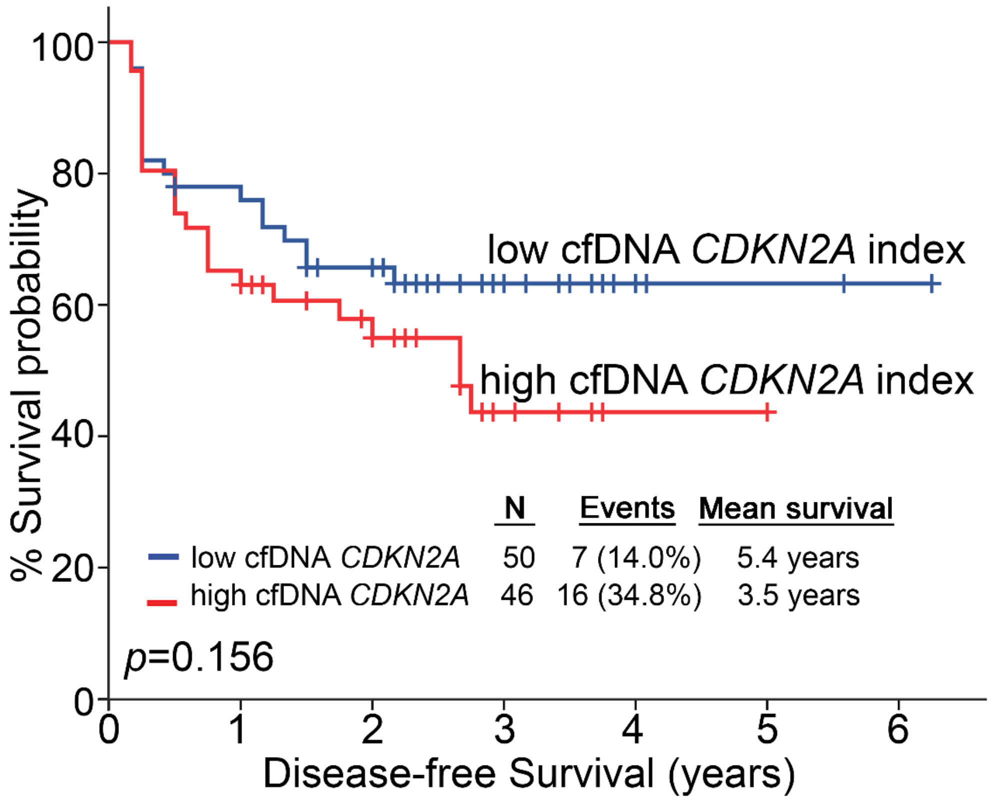

Figure S5. Kaplan-Meier survival curve for the disease-free survival (DFS) of NMIBC patients according to cfDNA *CDKN2A* index. *p*-values calculated by log-rank test. cfDNA: cell-free DNA.

Table S1. Cox regression analysis for the prediction of NMIBC (TaT1) patients' risk for relapse (DFS) and progression to invasive tumors (PFS) following TURBT according to tumor *CDKN2A* CNA status.

	Univariate analysis									
	Progression-free survival (PFS)					**Disease-free survival (DFS)**				
Covariant	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**
***CDKN2A* CNA**										
Loss	1.00					1.00				
Diploid/gain	2.834	1.068 – 7.523	0.037	1.242 – 12.516	0.018	2.168	1.129 – 4.163	0.020	1.229 – 4.610	0.010
Tumor Stage										
Ta	1.00					1.00				
T1	1.685	0.773 – 3.669	0.189	0.732 – 3.971	0.168	1.371	0.783 – 2.402	0.270	0.795 – 2.373	0.267
Tumor Grade										
Low	1.00					1.00				
High	2.244	1.037 – 4.853	0.040	1.026 – 5.202	0.033	1.493	0.848 – 2.632	0.165	0.850 – 2.613	0.140
Age (continuous)	1.013	0.973 – 1.054	0.531	0.977 – 1.054	0.493	0.997	0.969 – 1.026	0.842	0.972 – 1.026	0.830
Gender										
Male	1.00					1.00				
Female	1.712	0.587 – 4.996	0.325	0.314 – 4.522	0.335	1.420	0.636 – 3.170	0.392	0.535 – 3.208	0.390
	Multivariate analysis[e]									
Covariant	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**
***CDKN2A* CNA**										
Loss	1					1				
Diploid/gain	3.069	1.119 – 8.416	0.029	1.256 – 12.636	0.017	2.320	1.155 – 4.657	0.018	1.229 – 5.844	0.013
Tumor Stage										
Ta	1					1				
T1	0.678	0.199 – 2.313	0.535	0.177 – 2.155	0.512	1.038	0.438 – 2.461	0.933	0.365 – 2.838	0.945
Tumor Grade										
Low	1					1				
High	3.526	1.014 – 12.266	0.048	1.235 – 16.680	0.032	1.405	0.582 – 3.390	0.450	0.478 – 4.634	0.517
Age (continuous)	1.023	0.979 – 1.070	0.311	0.980 – 1.083	0.308	1.000	0.970 – 1.031	0.992	0.971 – 1.031	0.990
Gender										
Male	1					1				
Female	1.200	0.347 – 4.154	0.774	0.000 – 4.301	0.764	1.001	0.416 – 2.408	0.998	0.316 – 2.454	0.995

[a] Hazard Ratio, [b] 95% confidence interval of the estimated HR, [c] calculated by test for trend. Bootstrap *p*-value is based on 1000 bootstrap samples, [d] Bootstrap bias-corrected and accelerated 95% confidence interval of the estimated HR based on 1000 bootstrap samples, [e] Multivariate analysis adjusted for *CDKN2A* CNA status, tumors' stage, tumors' grade, patients' age and gender

Table S2. Cox regression analysis for the prediction of MIBC (T2-T4) patients' risk for relapse (DFS) and overall survival (OS) following RC according to tumor *CDKN2A* CNA status.

	Univariate analysis									
	Disease-free survival (DFS)					**Overall survival (OS)**				
Covariant	**HR**[a]	**95% CI**[b]	***p*-value**[c]	**Bootstrap BCa 95% CI**[d]	**Bootstrap *p*-value**[c]	**HR**[a]	**95% CI**[b]	***p*-value**[c]	**Bootstrap BCa 95% CI**[d]	**Bootstrap *p*-value**[c]
***CDKN2A* CNA**										
Loss	1.00					1.00				
Diploid/gain	0.957	0.528 – 1.734	0.885	0.536 – 1.651	0.864	0.784	0.419 – 1.469	0.448	0.421 – 1.511	0.451

[a] Hazard Ratio, [b] 95% confidence interval of the estimated HR, [c] calculated by test for trend. Bootstrap *p*-value is based on 1000 bootstrap samples,
[d] Bootstrap bias-corrected and accelerated 95% confidence interval of the estimated HR based on 1000 bootstrap samples

Table S3. Cox regression analysis for the prediction of NMIBC (TaT1) patients' risk for relapse (DFS) and progression to invasive tumors (PFS) following TURBT according to cfDNA *CDKN2A* index.

	Univariate analysis									
	Progression-free survival (PFS)					**Disease-free survival (DFS)**				
Covariant	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**
cfDNA *CDKN2A* index										
Low	1.00					1.00				
High	2.954	1.210 – 7.210	0.017	1.191 – 9.730	0.013	1.523	0.821 – 2.825	0.182	0.839 – 2.865	0.159
Tumor Stage										
Ta	1.00					1.00				
T1	1.262	0.557 – 2.861	0.577	0.531 – 3.274	0.580	1.051	0.568 – 1.943	0.875	0.546 – 1.914	0.870
Tumor Grade										
Low	1.00					1.00				
High	1.908	0.841 – 4.327	0.122	0.746 – 4.824	0.117	1.095	0.584 – 2.053	0.776	0.575 – 2.070	0.750
Age (continuous)	1.017	0.976 – 1.059	0.418	0.979 – 1.061	0.347	0.996	0.967 – 1.026	0.803	0.969 – 1.025	0.772
Gender										
Male	1.00					1.00				
Female	0.998	0.295 – 3.373	0.997	0.041– 3.008	0.997	1.380	0.609 – 3.128	0.440	0.519 – 3.081	0.424
	Multivariate analysis[e]									
Covariant	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**	**HR[a]**	**95% CI[b]**	***p*-value[c]**	**Bootstrap BCa 95% CI[d]**	**Bootstrap *p*-value[c]**
cfDNA *CDKN2A* index										
Low	1.00					1.00				
High	2.616	1.040 – 6.582	0.041	1.069 – 9.062	0.039	1.480	0.773 – 2.834	0.237	0.776 – 2.986	0.255
Tumor Stage										
Ta	1.00					1.00				
T1	0.680	0.207 – 2.235	0.525	0.121– 2.151	0.497	1.039	0.433 – 2.494	0.932	0.302 – 3.298	0.951
Tumor Grade										
Low	1.00					1.00				
High	2.632	0.799 – 8.665	0.111	0.995 – 14.902	0.082	0.971	0.400 – 2.357	0.947	0.316 – 3.055	0.954
Age (continuous)	1.021	0.976 – 1.068	0.371	0.971 – 1.075	0.354	0.996	0.967 – 1.026	0.797	0.968 – 1.027	0.782
Gender										
Male	1.00					1.00				
Female	0.902	0.262– 3.109	0.870	0.000 – 3.379	0.830	1.240	0.541– 2.846	0.611	0.407– 3.183	0.599

[a] Hazard Ratio, [b] 95% confidence interval of the estimated HR, [c] calculated by test for trend. Bootstrap *p*-value is based on 1000 bootstrap samples, [d] Bootstrap bias-corrected and accelerated 95% confidence interval of the estimated HR based on 1000 bootstrap samples, [e] Multivariate analysis adjusted for cfDNA *CDKN2A* index, tumors' stage, tumors' grade, patients' age and gender

Table S4. Cox regression analysis for the prediction of MIBC (T2-T4) patients' risk for relapse (DFS) and overall survival (OS) following RC according to cfDNA *CDKN2A* index.

	Univariate analysis									
	Disease-free survival (DFS)					**Overall survival (OS)**				
Covariant	**HR**[a]	**95% CI**[b]	***p*-value**[c]	**Bootstrap BCa 95% CI**[d]	**Bootstrap *p*-value**[c]	**HR**[a]	**95% CI**[b]	***p*-value**[c]	**Bootstrap BCa 95% CI**[d]	**Bootstrap *p*-value**[c]
cfDNA *CDKN2A* index										
Low	1.00					1.00				
High	0.871	0.509 – 1.489	0.613	0.524 – 1.538	0.612	0.854	0.483 – 1.510	0.587	0.489 – 1.528	0.573

[a] Hazard Ratio, [b] 95% confidence interval of the estimated HR, [c] calculated by test for trend. Bootstrap *p*-value is based on 1000 bootstrap samples,
[d] Bootstrap bias-corrected and accelerated 95% confidence interval of the estimated HR based on 1000 bootstrap samples

Table S5. Quality control and coverage metrics of DNA-seq PDX samples

PDX samples	Total number of reads	Mean coverage depth (fold)	Coverage within target region (%)	MAPD[a]
1	1883203	2518.87	99.72	0.39441
2	2060433	2784.86	99.93	0.42698
3	2044401	2744.52	98.15	0.47766
4	915206	1289.31	99.71	0.49905
5	1772378	2389.49	100.00	0.41090
6	322959	468.93	98.09	0.58995
7	1524642	2056.76	93.01	0.47898
8	1752553	2361.70	100.00	0.38851
9	1911000	2568.20	100.00	0.39664
10	2525261	3332.68	100.00	0.40899
11	663778	896.01	99.93	0.43910
12	3448397	4558.24	100.00	0.41968
13	1305438	1810.33	100.00	0.47777
14	2375262	3120.10	99.20	0.44610
15	2684208	3549.67	100.00	0.41497

[a] MAPD: median absolute pairwise difference