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Supplemental Information

***DANCR* Promotes Metastasis and Proliferation in Bladder Cancer Cells by Enhancing IL-11-STAT3 Signaling and CCND1 Expression**

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Supplementary figure

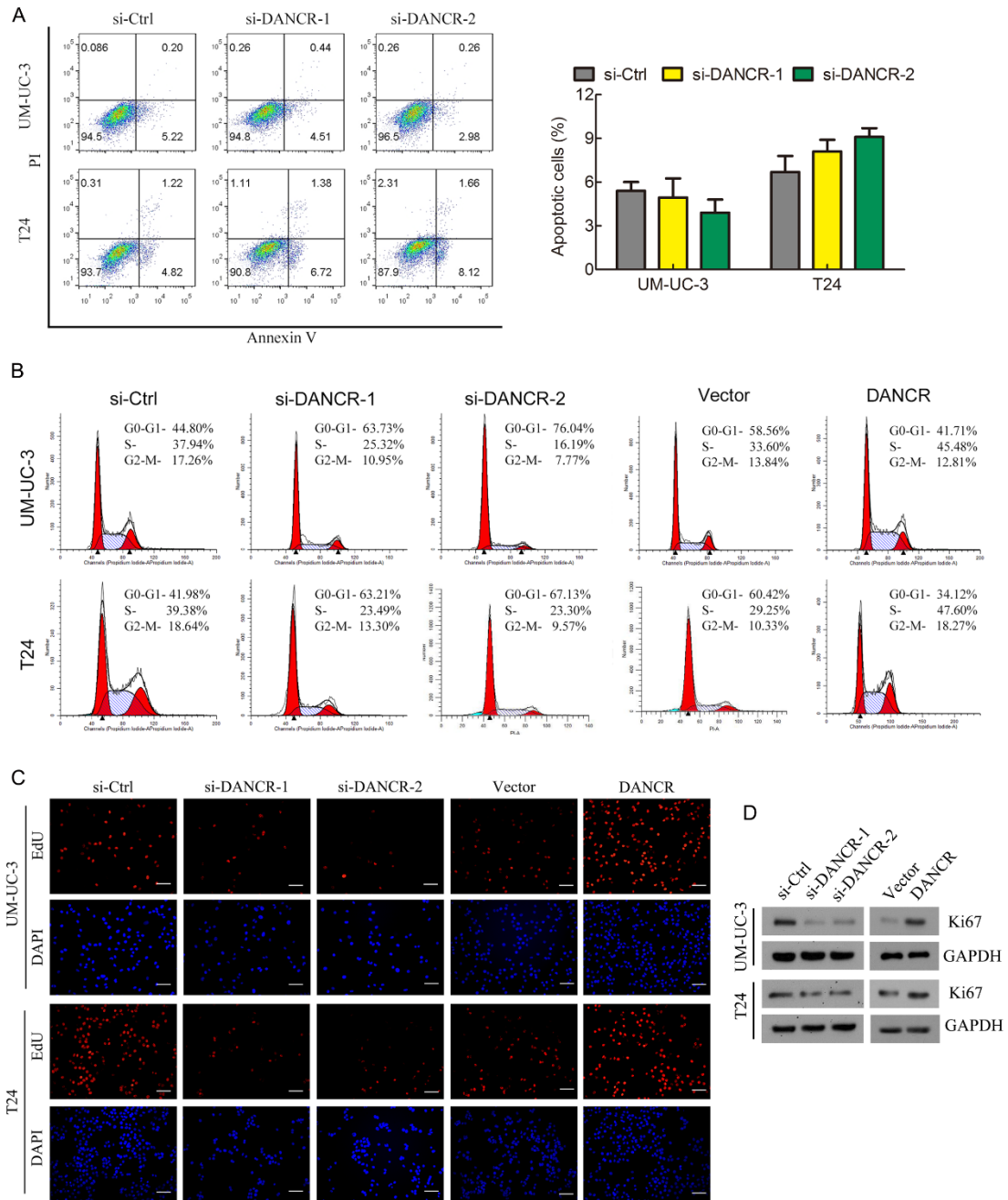


Figure S1. *DANCR* enhances the proliferation of bladder cancer cells *in vitro*

(A) The apoptosis analysis of *DANCR*-knockdown and control cells was performed after 48h transfection. The histogram shows the percentage (%) of apoptotic cells. (B) Representative images of flow cytometric analysis of T24 and UM-UC-3 cells transfected with *DANCR* siRNA or stably overexpressing *DANCR* compared with the corresponding control cells, as indicated. (C) Representative images of EdU assay measurement of the proportion of the cell population in the S phase. Blue, nucleus;

red, S-phase cells. Scale bars: white, 100 μm . **(D)** The expression of Ki67 was detected by western blotting. GAPDH was used as the internal control.

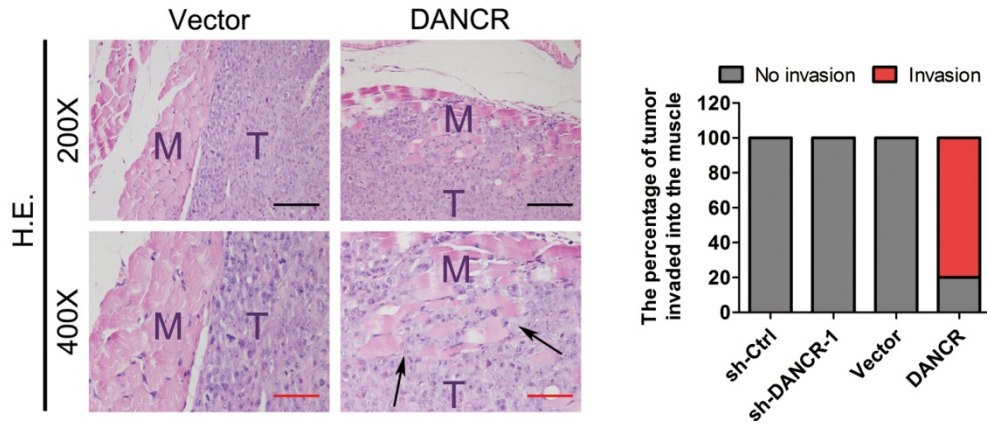


Figure S2. Representative images showing that tumors invaded into the surrounding muscle in *DANCR* overexpression groups and respective controls. M represents muscle and T represents tumor. The arrow indicates the invasive tissues. Histogram shows the percentage of the tumor invade into the surrounding muscle in *DANCR* knockdown or overexpression groups and the control group. Scale bars: red, 50 μm . black, 200 μm .

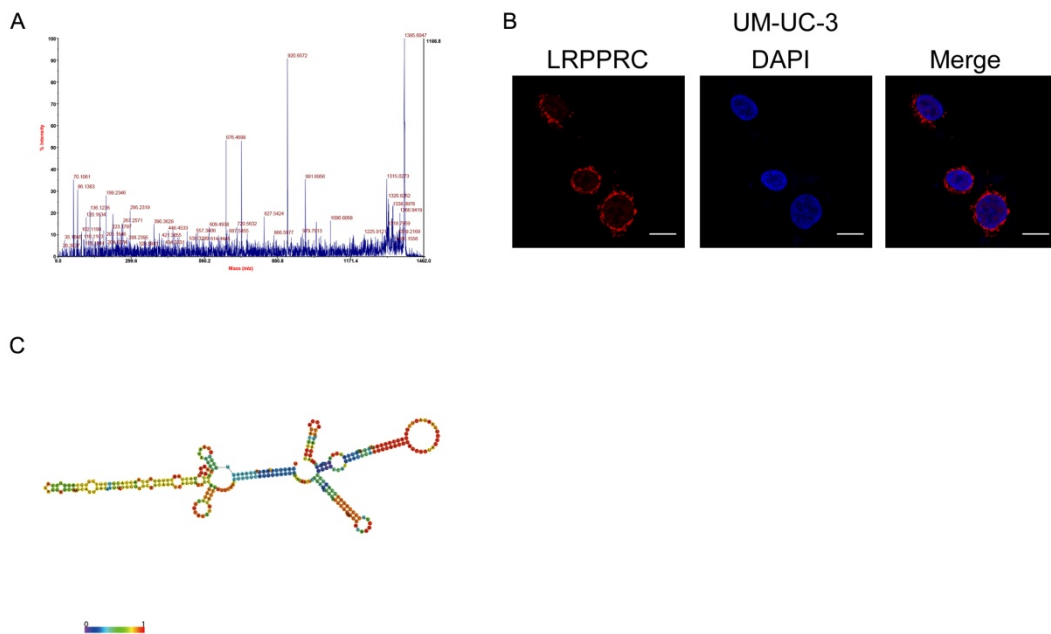


Figure S3. *DANCR* directly interacts with LRPPRC to play key roles in bladder cancer

(A) MS/MS profiles of target band (corresponding peptide sequences of LRPPRC) retrieved by *DANCR*. (B) The subcellular distribution of LRPPRC was visualized by immunofluorescence in UM-UC-3 cells. Scale bars: white, 10 μ m. (C) Prediction of 350–670 nt *DANCR* structure was based on minimum free energy (MFE) and partition function (<http://rna.tbi.univie.ac.at/>).

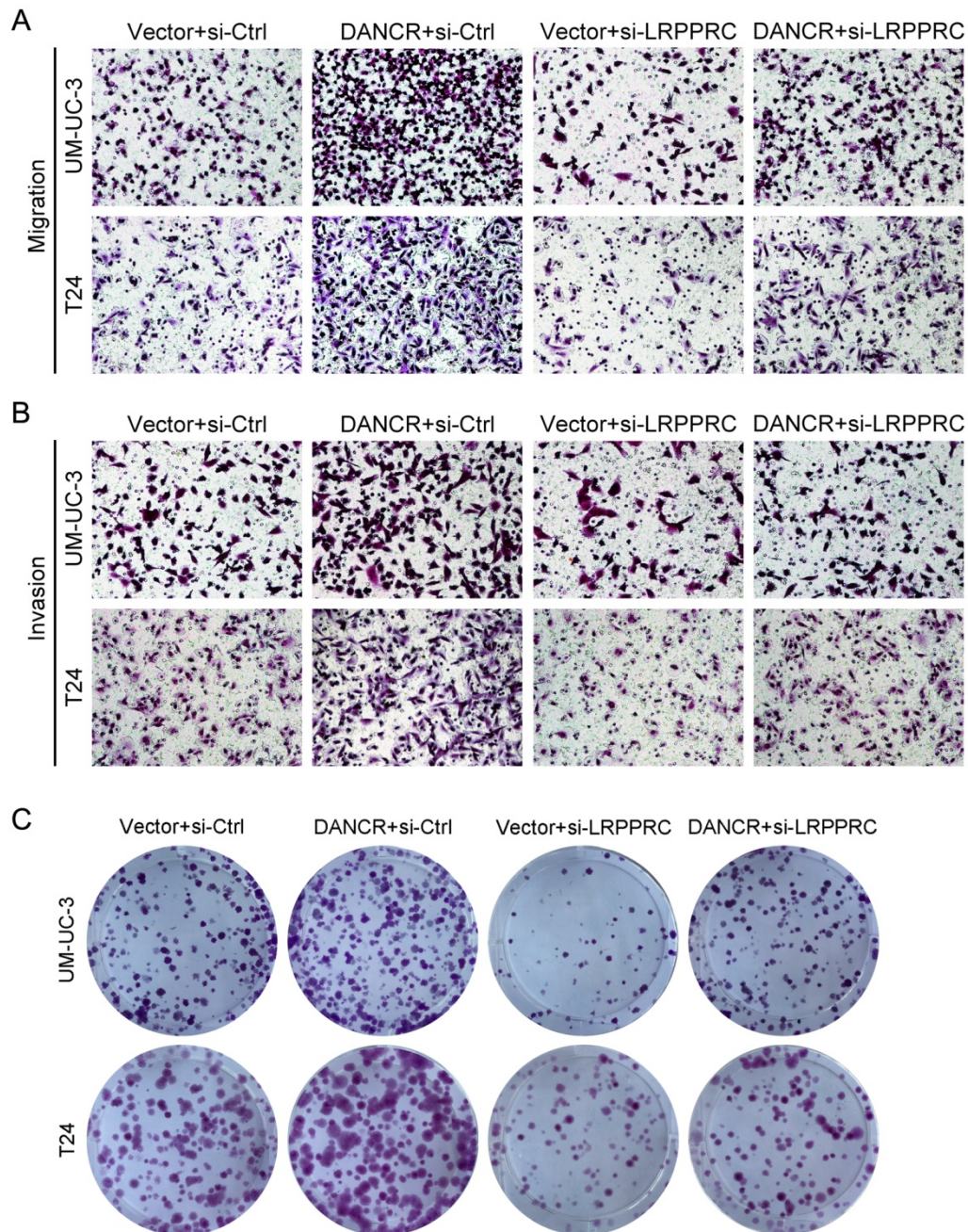


Figure S4. *DANCR* regulates metastasis and proliferation of BCa cells in an LRPPRC-dependent manner.

(A, B) Representative images of migration and invasion assays using *DANCR* overexpression or control cells combined with LRPPRC knockdown. (C) Representative images of colony formation were analyzed using *DANCR* overexpression or control cells combined with LRPPRC knockdown.

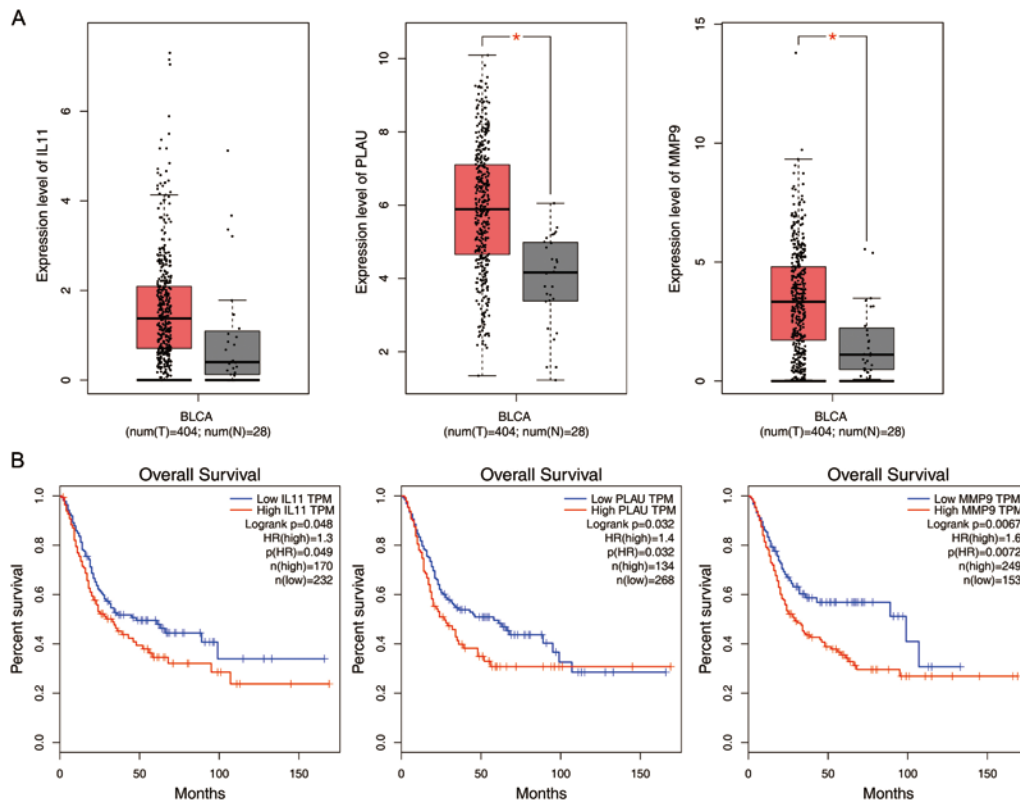


Figure S5. IL-11, PLAU and MMP9 were upregulated in BCa tissues and correlated positively with poor overall survival in BCa from TCG) cohort.

(A) The expression of IL-11, PLAU and MMP9 from the TCGA databases was analyzed in BCa patients. (B) Kaplan-Meier survival analysis of OS in BCa patients with expression profile of IL-11-high vs IL-11-low in TCGA databases. The similar analysis was performed in PLAU and MMP9. The data was obtained from GEPIA (<http://gepia.cancer-pku.cn/index.html>). The log-rank (Mantel-Cox) test was used to calculate p-values. $p < 0.05$ was considered statistically significant.

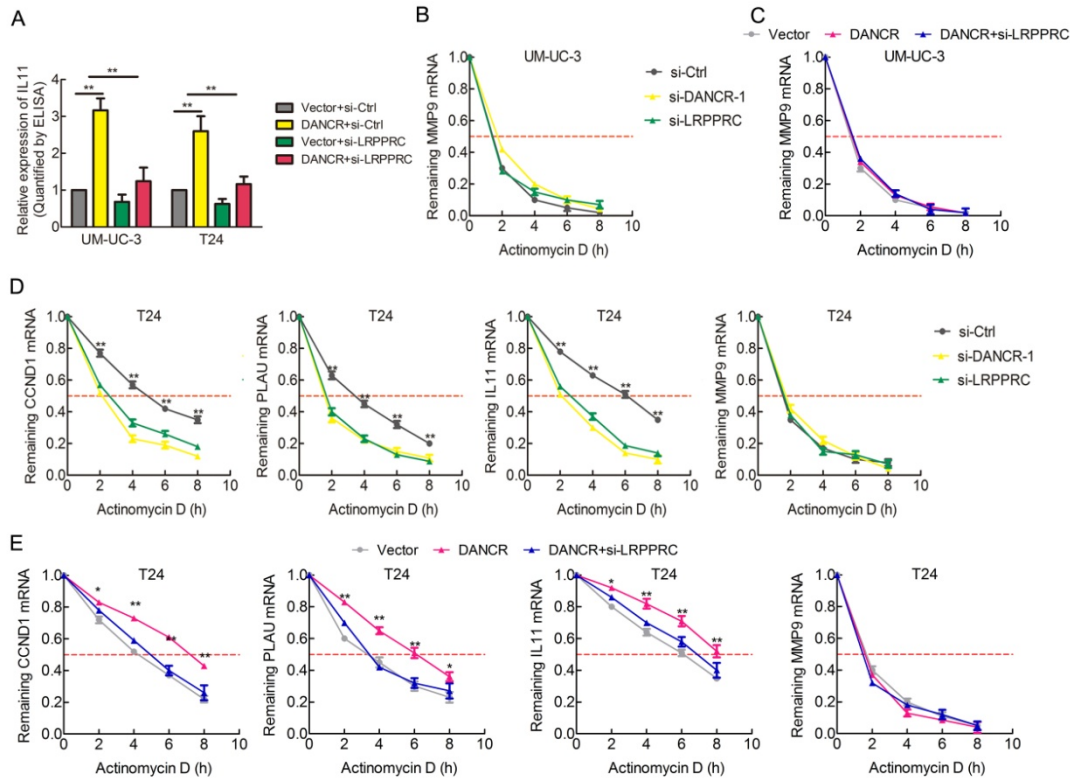


Figure S6. DANCR regulates mRNA stability via guiding LRPPRC to target genes.

(A) The secreted IL-11 expression was detected in *DANCR* overexpression or control cells combined with LRPPRC knockdown using ELISA. (B, D) UM-UC-3 and T24 cells expressing control siRNA, *DANCR* siRNA-1 or LRPPRC siRNA were treated with actinomycin D (5 μ g/mL) for the indicated periods of time. (C, E) UM-UC-3 and T24 cells stably expressing control, *DANCR* or *DANCR*+LRPPRC siRNA were treated with actinomycin D (5 μ g/mL) for the indicated periods of time. Total RNA was purified and then analyzed using qRT-PCR to examine the mRNA half-life of *CCND1*, *PLAU*, *IL11* and *MMP9*. * $p < 0.05$, ** $p < 0.01$.

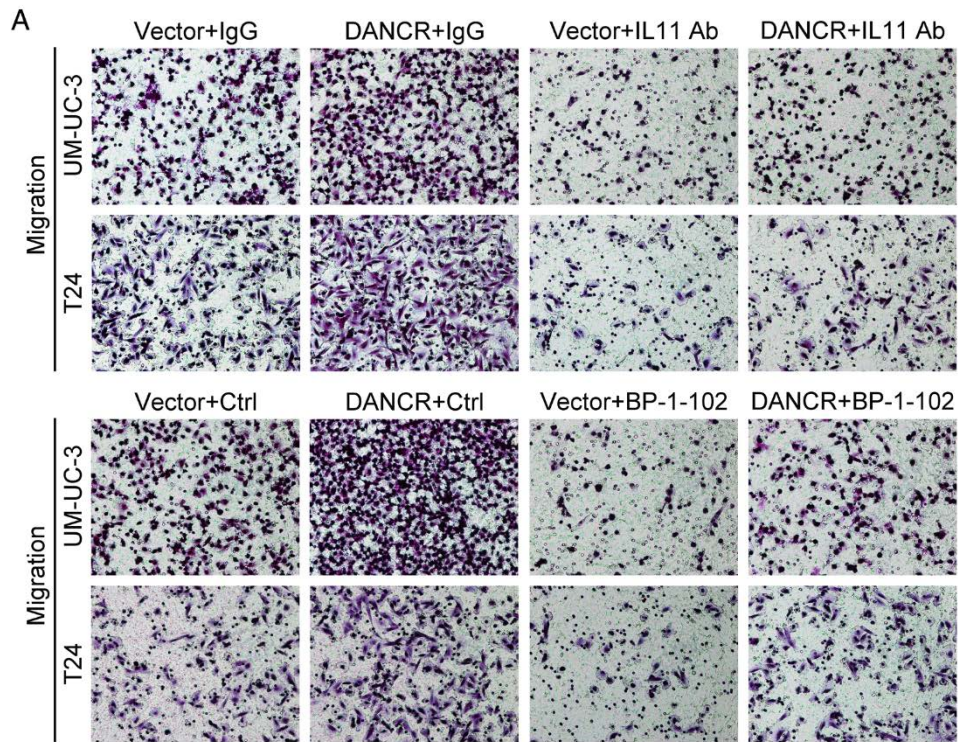


Figure S7. *DANCR* regulates metastasis of BCa cells in an IL-11-STAT3 signaling pathway-dependent manner.

(A) Representative images of migration and invasion assays using *DANCR* overexpression or control cells combined with anti-IL-11 antibody or STAT3 inhibitor (BP-1-102).

Supplementary Table 1. Correlation between *DANCR* expression and clinicopathological characteristics of bladder cancer patients.

Characteristic	Patient frequency	<i>DANCR</i>		Pearson Chi-square	<i>p</i> value
		Low	High		
Total	120	60	60		
Gender					
Male	94(78.3%)	47	47	0.000	1.000
Female	26(21.7%)	13	13		
Age					
≤65	67(55.8%)	39	28	4.089	0.043
>65	53(44.2%)	21	32		
Tumor number					
Single	70(58.3%)	33	37	0.730	0.393
Multiple	50(41.7%)	27	23		
Tumor size					
≤3cm	53(44.2%)	28	25	0.304	0.581
>3cm	67(55.8%)	32	35		
Pathologic tumor stage					
Ta-1	55(45.8%)	44	11	36.554	<0.001
T2-4	65(54.2%)	16	49		
Pathologic tumor grade					
Low	23(19.2%)	16	7	4.357	0.037
High	97(80.8%)	44	53		
Pathologic L.N. status					
N(-)	89(74.2%)	54	35	15.701	<0.001
N(+)	31(25.8%)	6	25		

Supplemental table 2 Univariate and multivariate analysis of factors associated with disease-free survival in bladder cancer.

Variable	Univariate			Multivariate		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Age, years (>65/≤65)	1.197	0.681–2.105	0.532			NA
Gender (female/male)	0.458	0.195–1.076	0.073			NA
Histological grade (High/Low)	1.480	0.714–3.067	0.292			NA
Tumor stage (T2–T4/Ta–T1)	2.567	1.409–4.675	0.002	1.428	0.626–3.255	0.397
Nodal metastasis (N1–N2/N0)	2.420	1.343–4.361	0.003	1.567	0.788–3.116	0.200
Tumor size (>3 cm/≤ 3 cm)	1.264	0.719–1.264	0.415			NA
Tumor number (multiple/single)	0.829	0.465–1.478	0.525			NA
<i>DANCR</i> (high/low)	2.642	1.468–4.754	0.001	1.879	0.906–3.899	0.090

Univariate and multivariate analysis. Cox proportional hazards regression model. Variables associated with survival by univariate analyses were adopted as covariates in multivariate analyses. Significant P-values are shown in bold font. HR > 1, risk for death increased; HR < 1, risk for death reduced.

Supplemental table 3 The number of metastatic LNs in UM-UC-3 xenograft mice.

	No. total LNs	No. metastatic LNs	Metastatic ratio (%)
sh-Ctrl	10	5	50.0
sh- <i>DANCR</i> -1	10	0	0
Vector	10	4	40.0
<i>DANCR</i>	10	9	90.0

Supplementary Table 4. Primers used in this study.

Primer Name	Sequence 5'-3'
DANCR Forward	TCGGAGGTGGATTCTGTTAGG
DANCR Reverse	TCGGTGTAGCAAGTCTGGTGA
GAPDH Forward	CAAGGCTGAGAACGGGAAG
GAPDH Reverse	TGAAGACGCCAGTGGACTC
DANCR(1-350) Forward	GCCCTTGCCCAGAGTCTTCCCCG
DANCR(1-350) Reverse	AGGGATAGTTGGCTTAAGTCAATTGAA
DANCR(1-700) Forward	GCCCTTGCCCAGAGTCTTCCCCG
DANCR(1-700) Reverse	TCCCCCGTGCCACCCAGAGGG
DANCR(320-915) Forward	GTATTTCAATTGACTTAAGCCAATA
DANCR(320-915) Reverse	GTCAGGCCAAGTAAGTTTATTAACCT
DANCR(670-915) Forward	TACACCGAAGCCCTCTGGGTGG
DANCR(670-915) Reverse	GTCAGGCCAAGTAAGTTTATTAACCT
LRPPRC Forward	GAGAGATGCCGGAATTGAGC
LRPPRC Reverse	CTCGGACTTCTCCACCTTCT
IL-11 Forward	TATGGGACAAAGCTGCAAGGT
IL-11 Reverse	GGTGGCGTTCTATCCACAGAT
PLAU Forward	CCGCATGACTTTGACTGGAAT
PLAU Reverse	GCCATTCTCTTCCTTGGTGTG
MMP-9 Forward	ACGCAGACATCGTCATCCAGT
MMP-9 Reverse	GGACCACAACCTCGTCATCGTC
CCND1 Forward	GCTGCGAAGTGGAAACCATC
CCND1 Reverse	CCTCCTTCTGCACACATTTGAA
U6 Forward	CTCGCTTCGGCAGCACATATAC
U6 Reverse	AACGCTTCACGAATTTGCGTGTC
MALAT1 Forward	GACGGAGGTTGAGATGAAGC
MALAT1 Reverse	ATTCGGGGCTCTGTAGTCCT

Supplementary Table 5. Sequences of siRNA oligos, shRNAs used in this study.

Name	Sequence 5'-3'
siRNA	
Si-Ctrl	UUCUCCGAACGUGUCACGUTT
Si-DANCR-1	GAGCUAGAGCAGUGACAAUTT
Si-DANCR-2	GCGUACUAACUUGUAGCAATT
Si-LRPPRC	GGAGGAGCAUUUGAGACAATT
shRNA	
Sh-Ctrl	CAACAAGATGAAGAGCACCAA
Sh-DANCR-1	AGGAGCTAGAGCAGTGACAAT

Supplementary Table 6. The probes used in this study.

Probe Name	Sequence 5'-3'	Label
Used in FISH		
DANCR-1	CGCGCAACTCCAGCTGACAA	5'- and 3'-CY3
DANCR-2	GTGAACATGAAGCACCTGCT	5'- and 3'-CY3
DANCR-3	TGCCAGGCTTCTCCACCAGT	5'- and 3'-CY3
U6- probe	CACGAATTTGCGTGTTCATCCTT	5'- and 3'-CY3
Used in RNA Pulldown		
DANCR-even-1	CGCCCGAAACCCGCTACATA	3'-Biotin
DANCR-even-2	TGCACTTCCGCAGACGTAAG	3'-Biotin
DANCR-even-3	CTTATTAGAGGCACTTTCCT	3'-Biotin
DANCR-even-4	GTGAACATGAAGCACCTGCT	3'-Biotin
DANCR-even-5	TTGAGTTAGCGGGGGCGGAG	3'-Biotin
DANCR-even-6	GTGCCACCCAGAGGGCTTCG	3'-Biotin
DANCR-even-7	TCATGACCGGCTTACAATAT	3'-Biotin
DANCR-even-8	TGCCAGGCTTCTCCACCAGT	3'-Biotin
DANCR-odd-1	CGCGCAACTCCAGCTGACAA	3'-Biotin
DANCR-odd-2	GGAGCTCAAGGTCGGCTGGG	3'-Biotin
DANCR-odd-3	ACAGGACATTCCAGCTTCAA	3'-Biotin
DANCR-odd-4	TGTGGCACTGCACGGACACG	3'-Biotin
DANCR-odd-5	GTCCCTAACAGAATCCACCT	3'-Biotin
DANCR-odd-6	TATAGCGCCTAGATAACGGT	3'-Biotin
DANCR-odd-7	ACAAGGGGGTGTAATCCACG	3'-Biotin
DANCR-odd-8	TTATATGGGGGAGAGAGACC	3'-Biotin
LacZ-1	CCAGTGAATCCGTAATCATG	3'-Biotin
LacZ-2	TCACGACGTTGTAAAACGAC	3'-Biotin
LacZ-3	ATTAAGTTGGGTAACGCCAG	3'-Biotin
LacZ-4	AGGTTACGTTGGTGTAGATG	3'-Biotin
LacZ-5	AATGTGAGCGAGTAACAACC	3'-Biotin
LacZ-6	GTAGCCAGCTTTCATCAACA	3'-Biotin
LacZ-7	AATAATTCGCGTCTGGCCTT	3'-Biotin
LacZ-8	AGATGAAACGCCGAGTTAAC	3'-Biotin

The full-length blots of manuscript are presented.

Figure 5C

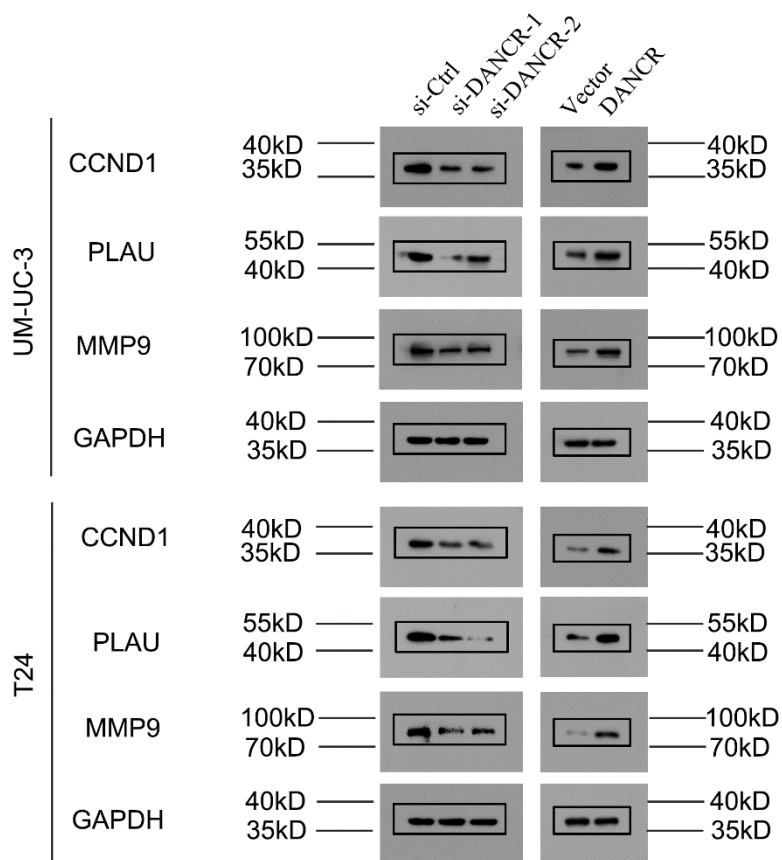


Figure 6C

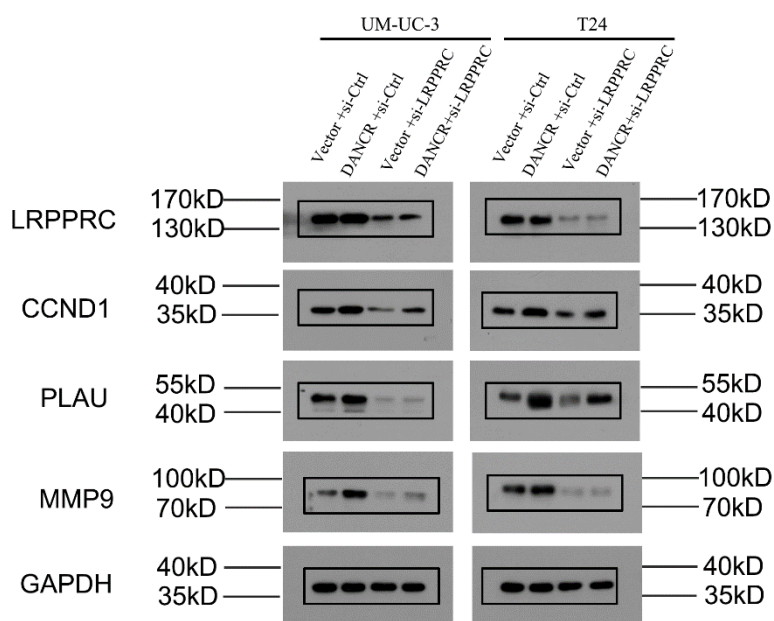


Figure 7G

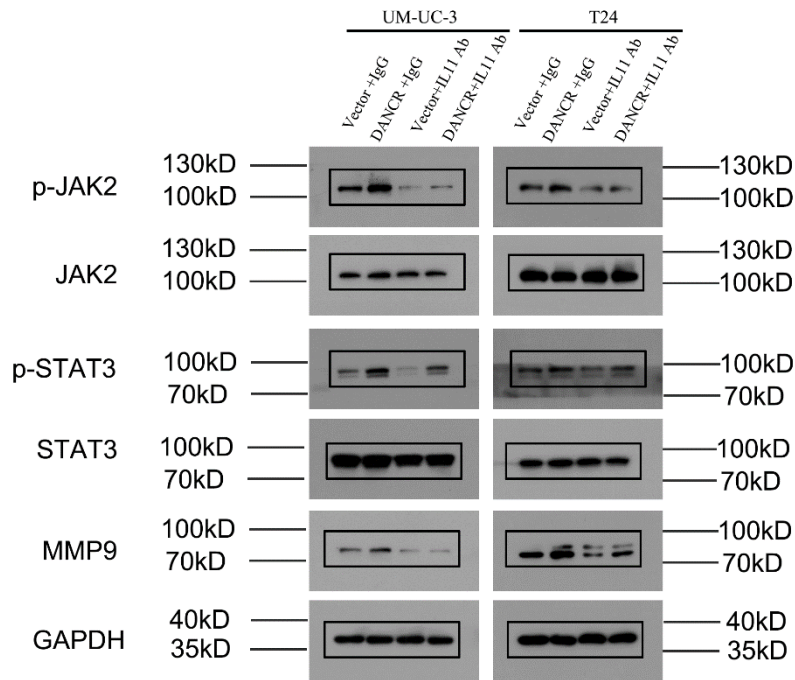


Figure 7H

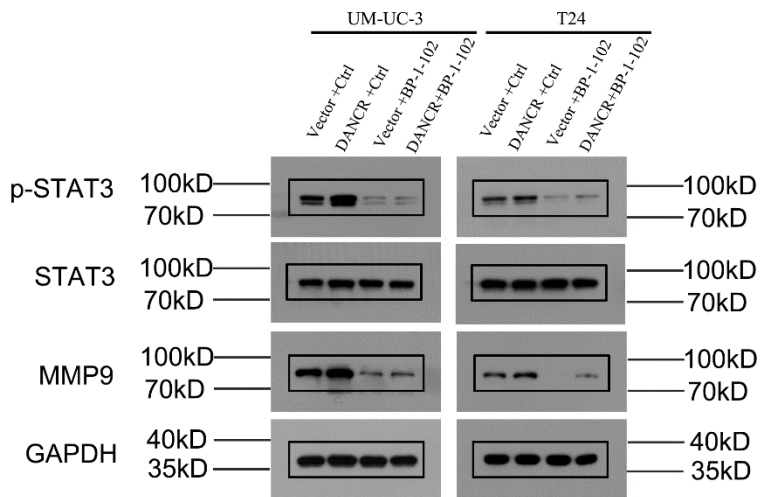


Figure S1D

