Silencing of *HJURP* induces dysregulation of cell cycle and ROS metabolism in bladder cancer cells via PPARγ-SIRT1 feedback loop

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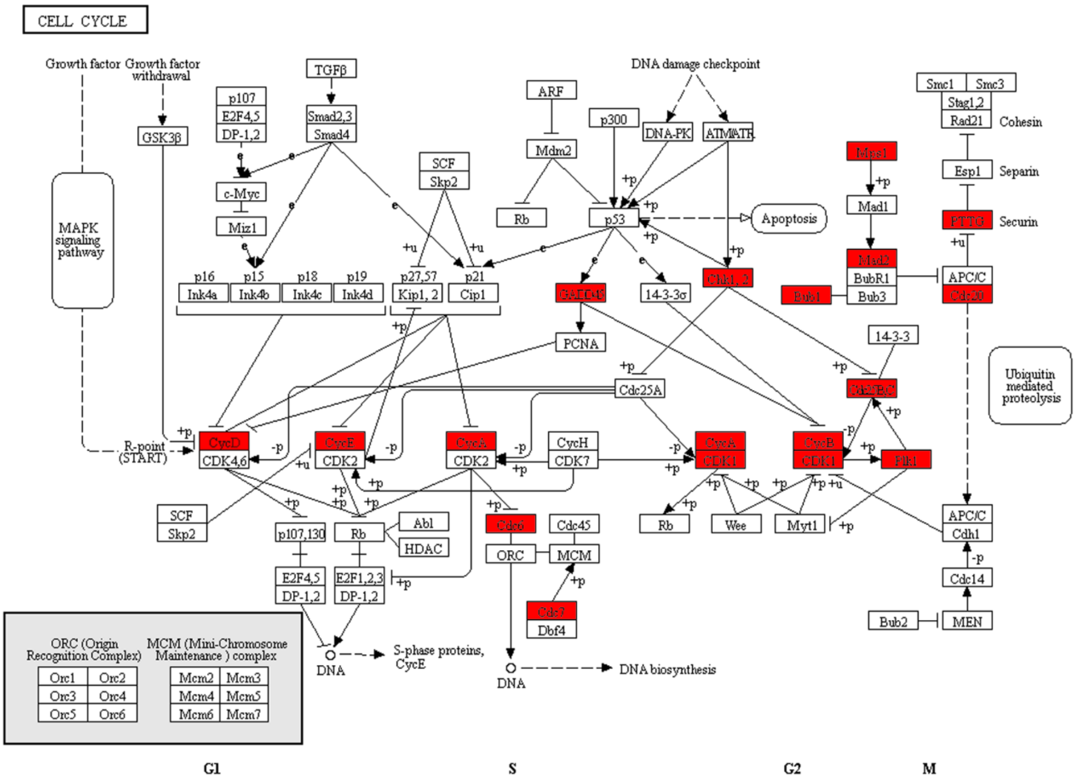
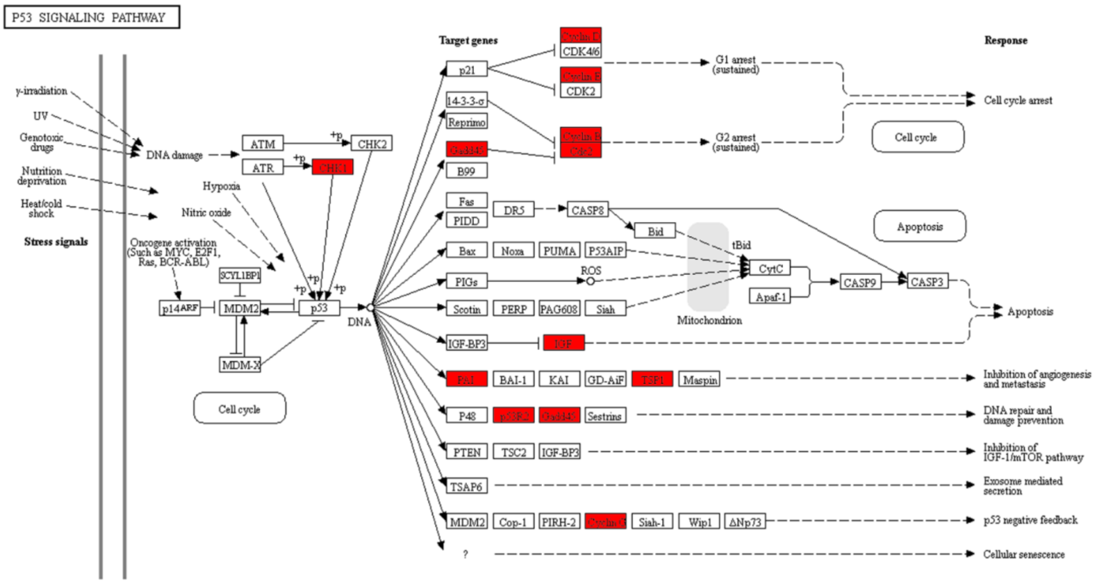
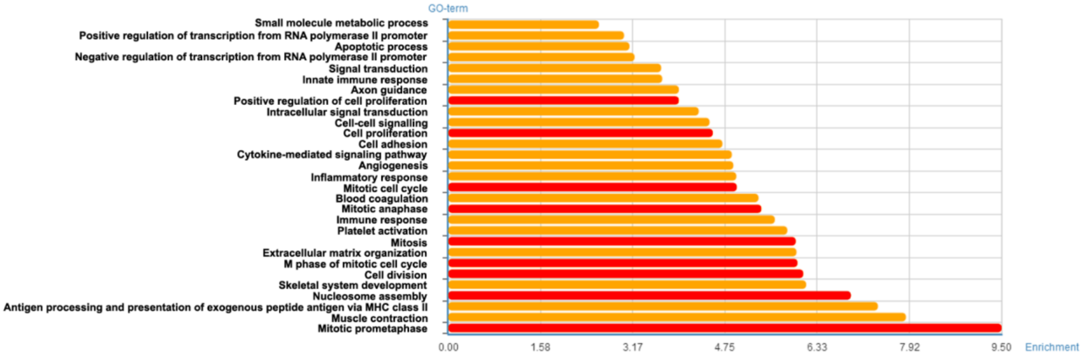
Supplementary Information 1



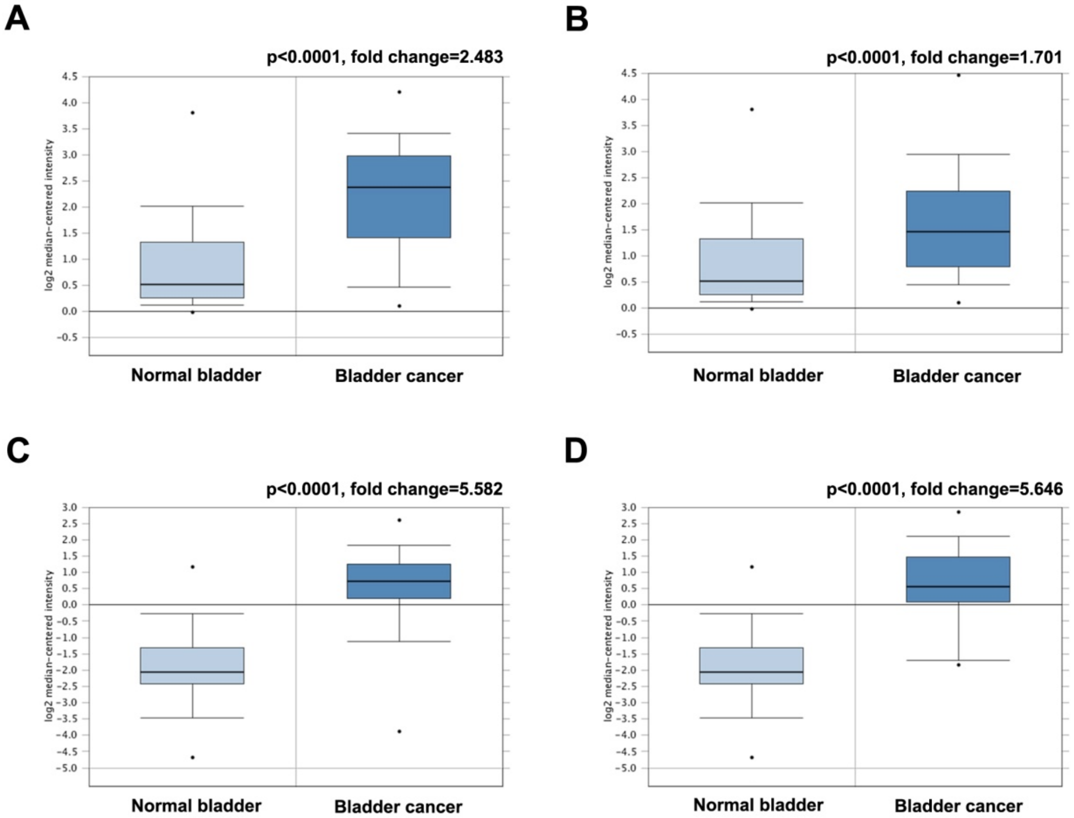
Supplementary Figure S1. Cell cycle pathway was affected in the BCa tissues compared with normal bladder tissues. Overrepresentation analysis using microarray raw data and DAVID database revealed altered genes (marked in red) involved in cell cycle pathway. The reference cell cycle pathway was from KEGG Pathway Database (http://www.kegg.jp/kegg/kegg1.html).



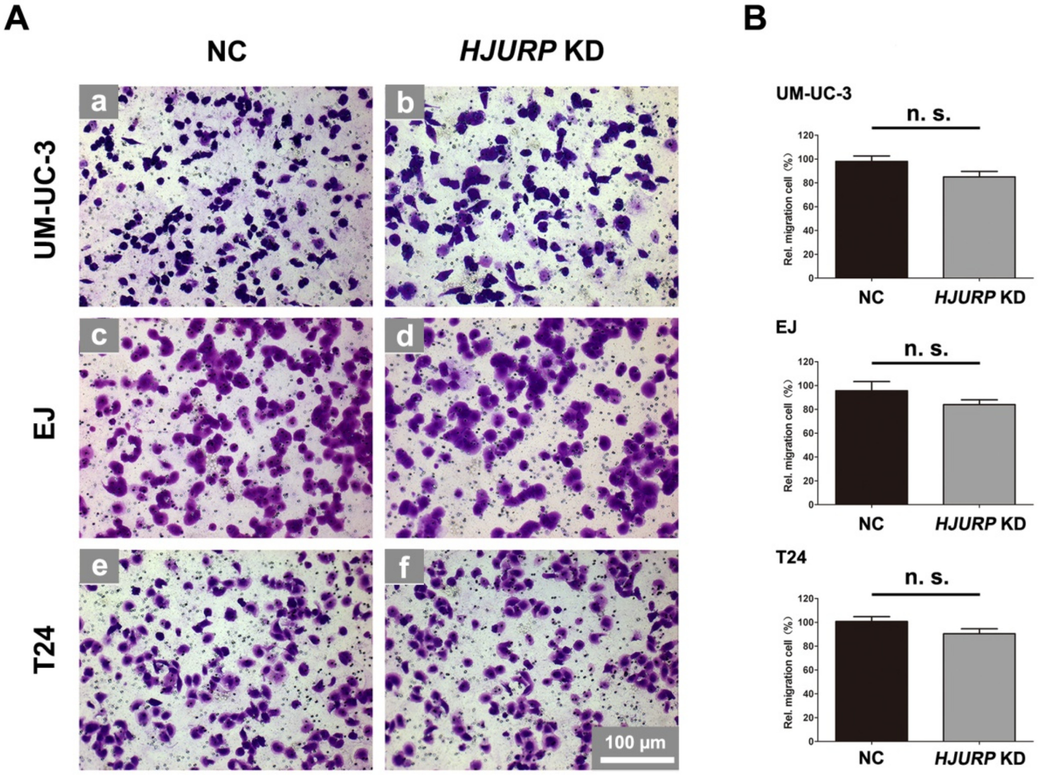
Supplementary Figure S2. The p53 signalling pathway was affected in the BCa tissues compared with normal bladder tissues. Overrepresentation analysis using microarray raw data and DAVID database revealed altered genes (marked in red) involved in p53 signalling pathway. The reference p53 signalling pathway was from KEGG Pathway Database (http://www.kegg.jp/kegg/kegg1.html).



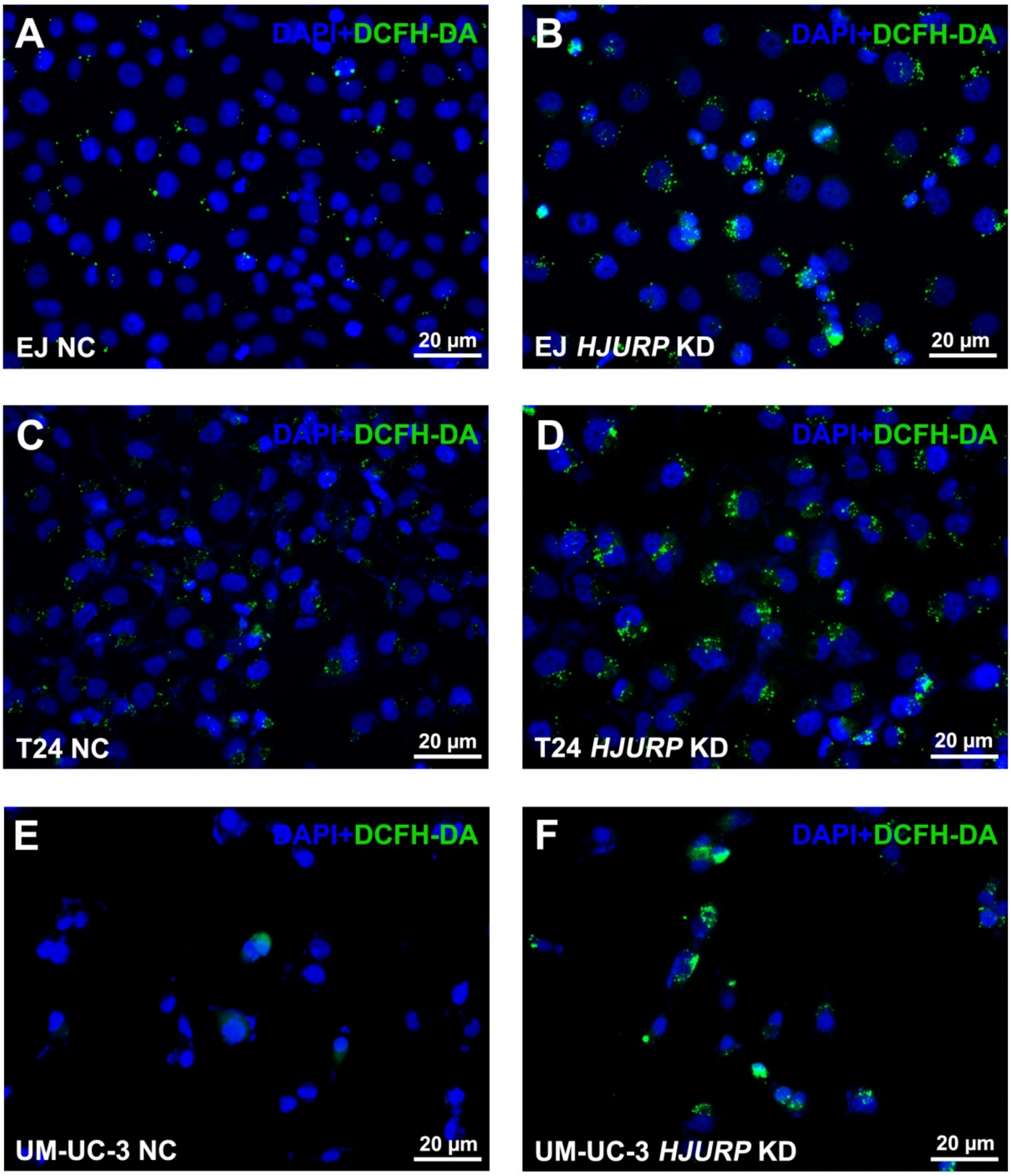
Supplementary Figure S3. Significantly changed cell functions related with bladder cancer by mRNA microarray using bladder cancer tissues versus normal bladder tissues (ranking according to enrichment score). According GO analysis using the mRNA microarray data, the alteration of mitotic prometaphase (ranked 1), nucleosome assembly (ranked 4), cell division (ranked 6), M phase of mitotic cell cycle (ranked 7), mitosis (ranked 9), mitoic anaphase (ranked 12), mitotic cell cycle (ranked 14), cell proliferation (ranked 19), positive regulation of cell proliferation (ranked 22) was shown in the human bladder cancer (marked in red). *HJURP* was involved in these functions especially in the nucleosome assembly.



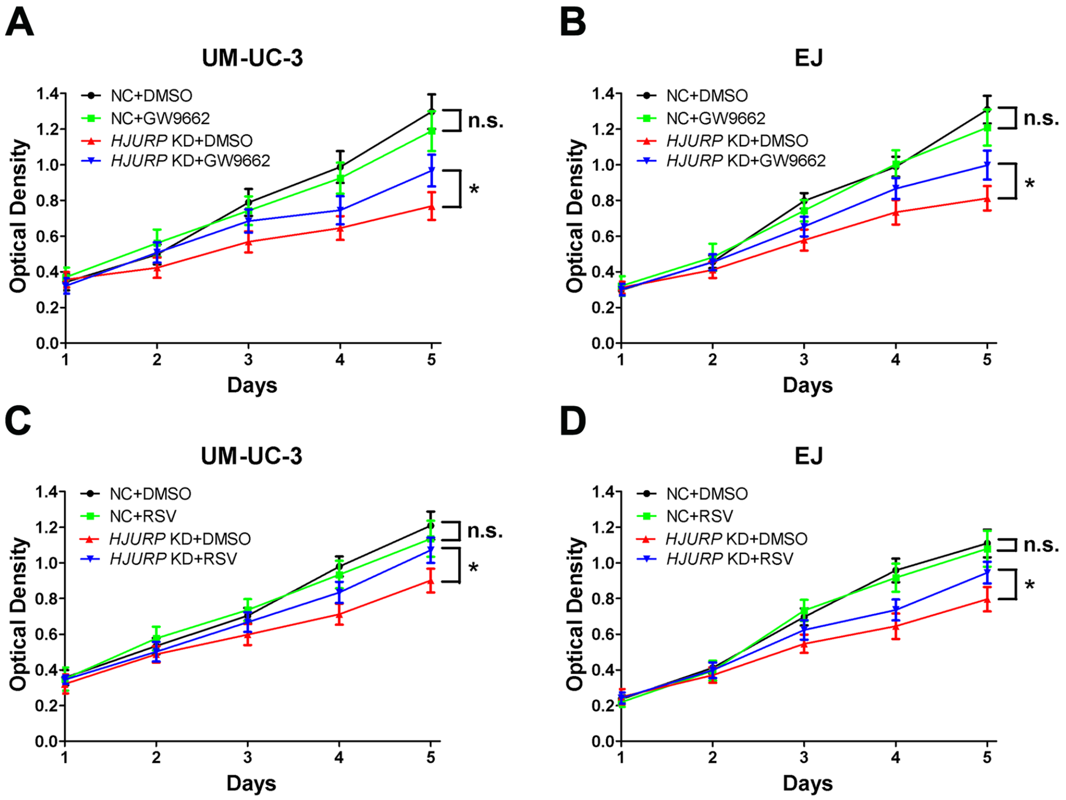
Supplementary Figure S4. Oncomine database confirmed overexpression of *HJURP* in bladder cancer. Oncomine database (www.oncomine.org) exhibited an upregulation of *HJURP* at transcriptional level in bladder cancer tissues compared with normal bladder tissues, revealed from four microarray results **(A-D)**. P value and fold changes were indicated.



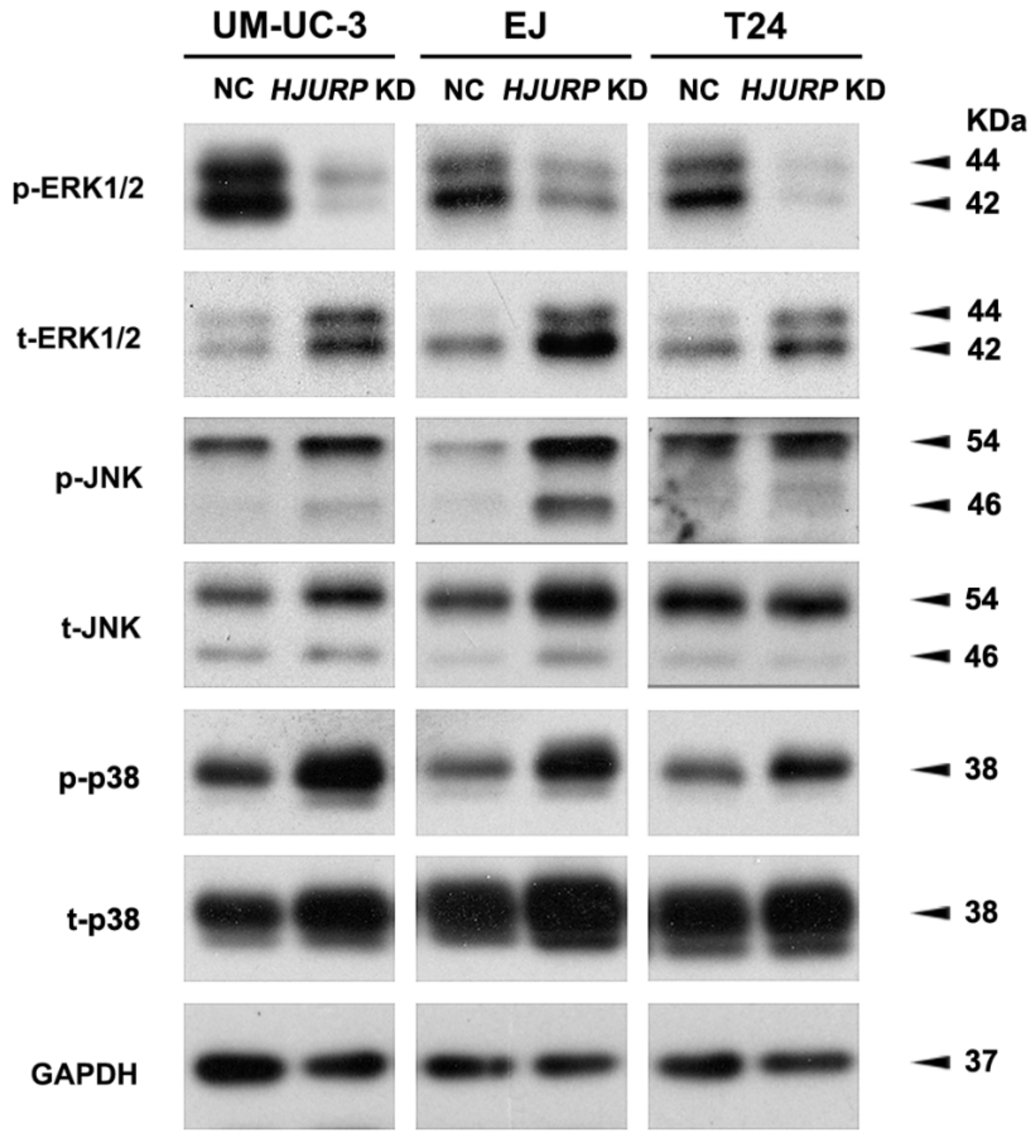
Supplementary Figure S5. The effect of *HJURP* on migration of BCa cells measured by transwell assay. (A) Distinct BCa cells, UM-UC-3 (a-b), EJ (c-d) and T24 (e-f), were transfected by siRNA to knockdown *HJURP* gene expression and cell migration was evaluated by trasnswell assay. **(B)** Statistical analysis of migration rate of BCa cells, suggesting no significantly effect of *HJURP* on migration of the BCa cells. The scale bar for A is 100 μm.



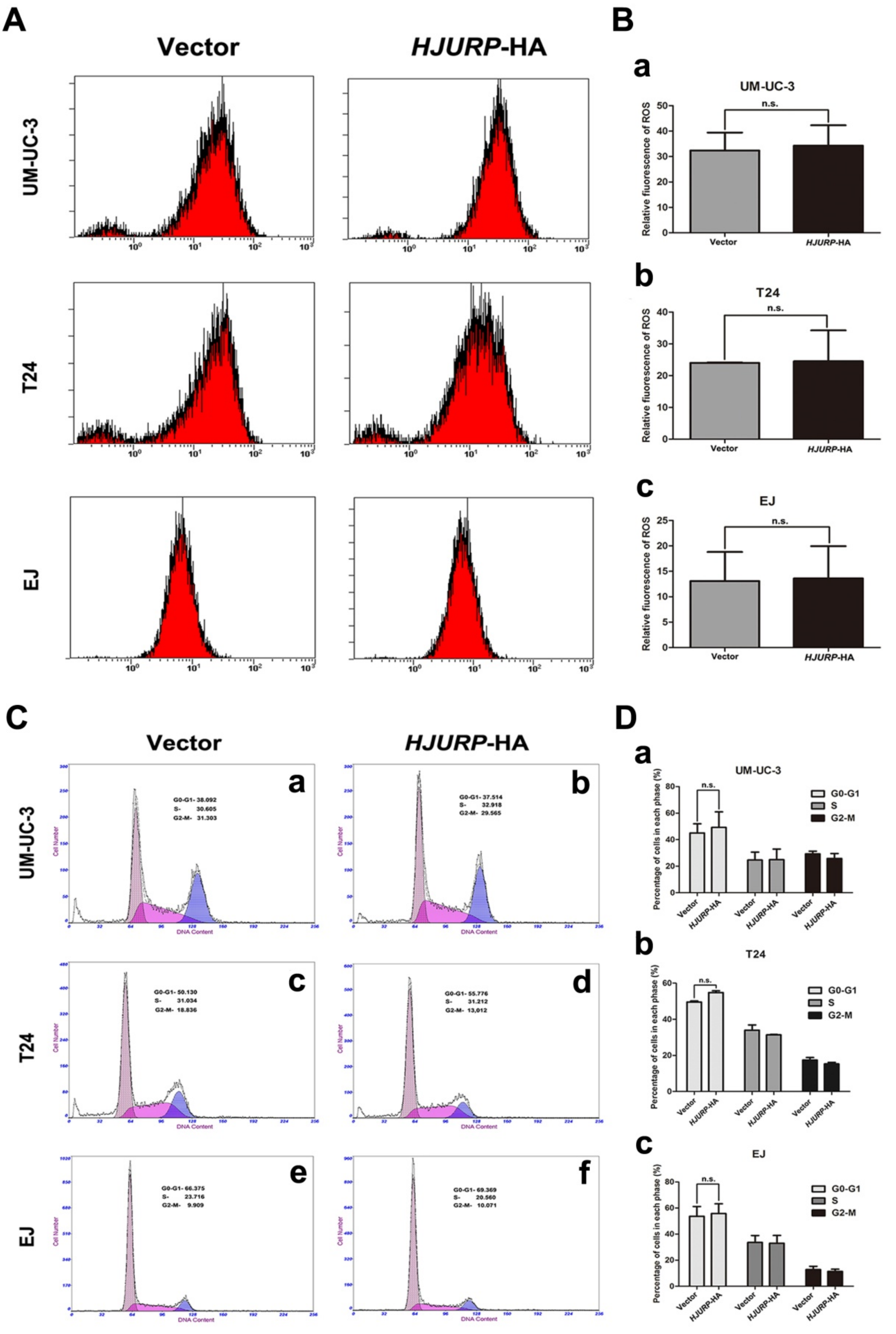
Supplementary Figure S6. The effect of *HJURP* on ROS status of BCa cells stained by DCFH-DA. Three BCa cells (EJ, T24 and UM-UC-3) were transfected either by negative control siRNA **(A, C, E)** or *HJURP*-target specific siRNA **(B, D, F)**. The cells with reduced *HJURP* showed significantly increased ROS status comparing with the control group. ROS was stained with DCFH-DA and nuclei were labelled with DAPI. Photos were taken by fluorescence microscope. The scale bars for A-F are 20 μm.



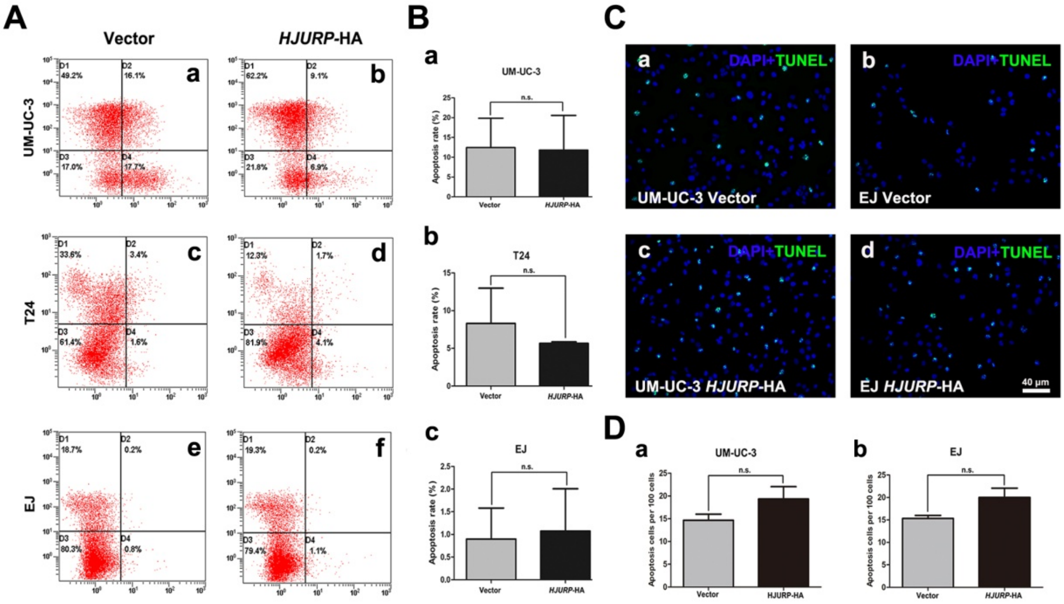
Supplementary Figure S7. Knockdown of *HJURP* inhibited proliferation of BCa cells via PPARγ/SIRT1 negative feedback loop. (A) UM-UC-3 and **(B)** EJ cells were pre-treated with indicated amount of GW9662 and vehicle DMSO for 12h. CCK-8 assay was used to detect the proliferation of *control-siRNA* (black and green line) and *HJURP-target-specific-siRNA* (violet and red line) BCa cells. After pre-treatment with appropriate amount of RSV and DMSO for 12h, UM-UC-3 **(C)** and EJ **(D)** transfected with NC (black and green line) or *HJURP* KD (violet and red line) were allowed to grow at the indicated times and the cell viability was determined by CCK-8 assay. Statistical analysis for CCK-8 assay revealed that RSV and GW9662 could rescue the proliferation inhibition of BCa cells caused by *HJURP* KD, but showed no significant (n. s.) alterations in the control group. All values shown were mean ± SD of triplicate measurements and repeated three times with similar results. * $p<0.05$.



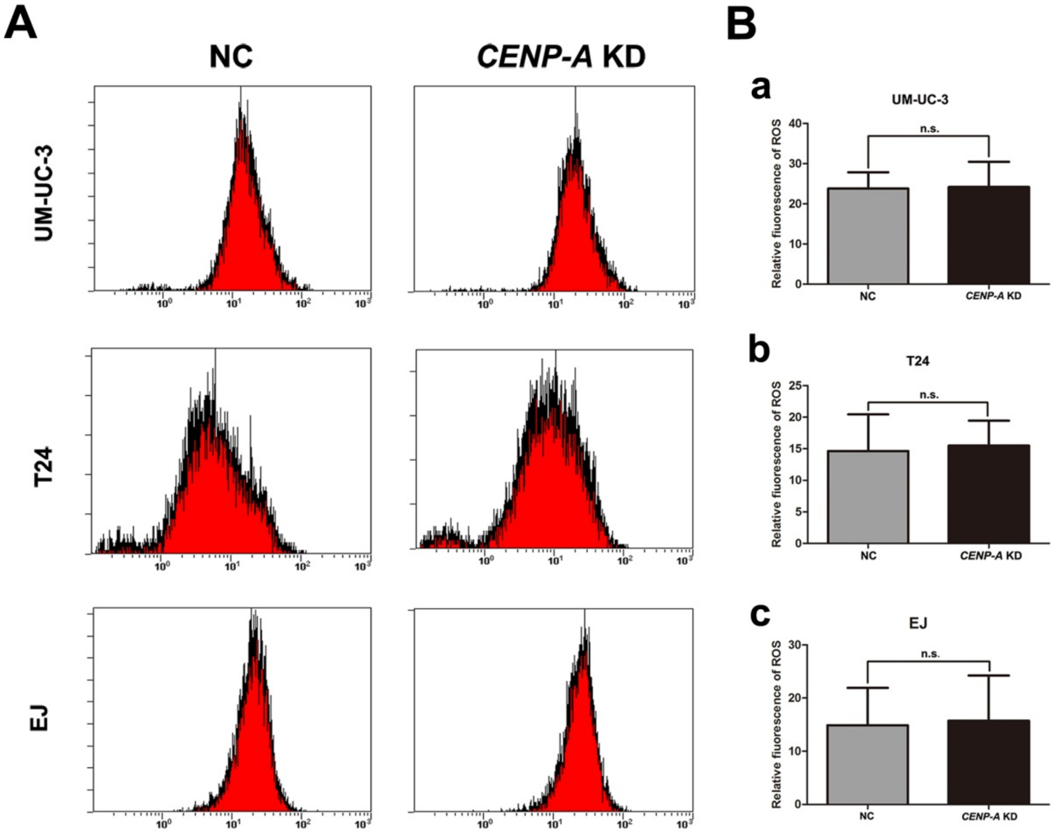
Supplementary Figure S8. Alteration of MAPK pathway in *HJURP*-decreased BCa cells. Western blot analysis of phosphorylated ERK1/2 (p-ERK1/2), total ERK1/2 (t-ERK1/2), phosphorylated JNK (p-JNK), total JNK (t-JNK), phosphorylated p38 (p-p38) and total p38 (t-p38) in the UM-UC-3, EJ and T24 cells by *siRNA* treatment. Protein abundance of GAPDH was used as a loading control.



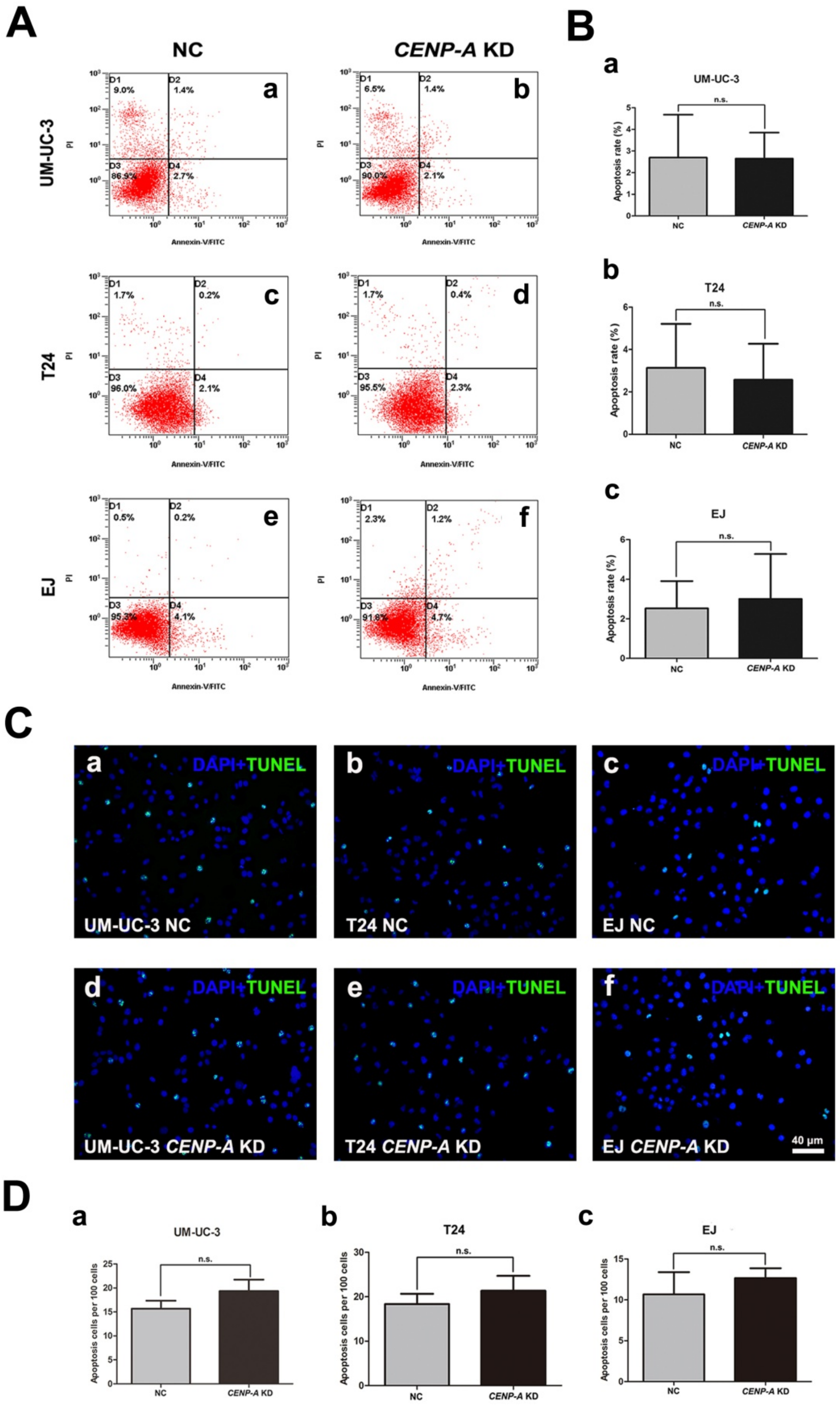
Supplementary Figure S9. ***HJURP*-overexpression exhibited no significant effect on ROS production and cell cycle regulation in the BCa cells.** **(A)** Flow cytometry analysis of ROS production in the *control* (Vector) and *pWSLV-11-HJURP* plasmid (*HJURP*-HA) transfected BCa cells (UM-UC-3, T24 and EJ). **(B)** Statistical analysis revealed no significant (n. s.) changes of ROS production by *HJURP*-overexpression. **(C)** Flow cytometry analysis for the alteration of percentages (%) of cell populations at different stages of cell cycles in the *control* (Vector) and *pWSLV-11-HJURP* plasmid (*HJURP*-HA) transfected BCa cells (UM-UC-3, T24 and EJ). **(D)** Statistical analysis showed no significant (n. s.) changes of percentages (%) of cell populations at different stages of cell cycles in UM-UC-3 (a), T24 (b) and EJ cells (c). All values shown were mean ± SD of triplicate measurements and repeated three times with similar results.



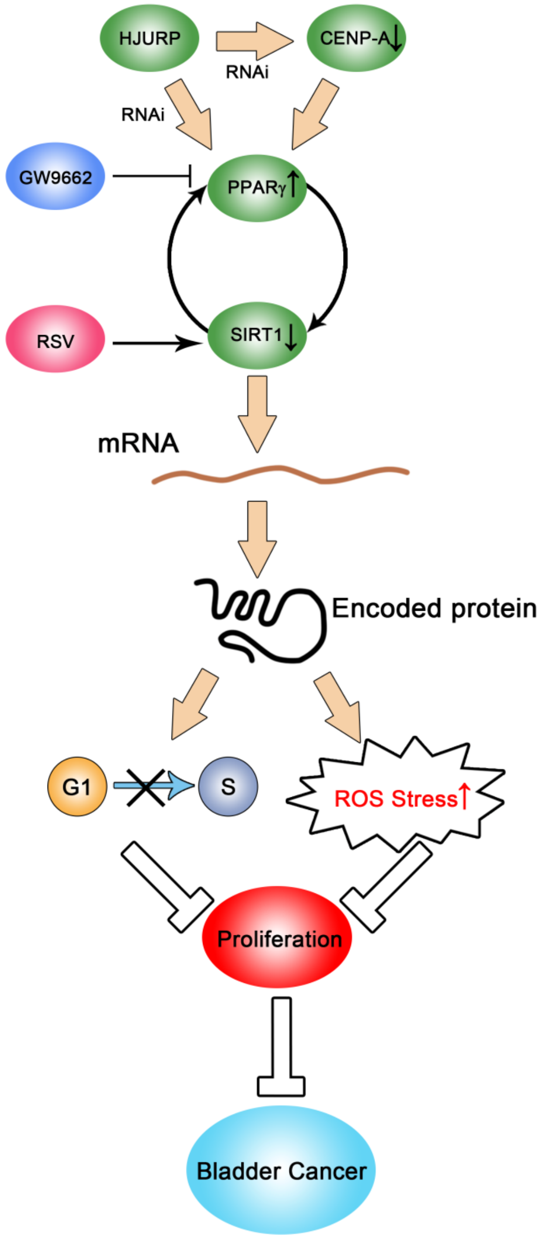
Supplementary Figure S10. The effect of *HJURP*-overexpression on bladder cancer cells apoptosis. (A) Flow cytometry analysis for apoptosis in the *control* (Vector) and *pWSLV-11-HJURP* plasmid (*HJURP*-HA) transfected BCa cells (UM-UC-3, T24 and EJ). **(B)** Statistical analysis revealed no significant (n. s.) changes of apoptotic rate by *HJURP*-overexpression. **(C)** TUNEL staining for the apoptotic cells in the *control* (Vector) and *pWSLV-11-HJURP* plasmid (*HJURP*-HA) transfected BCa cells (UM-UC-3, EJ). The scale bar for C is 40 μm. **(D)** Statistical analysis for TUNEL assay revealed no significant (n. s.) apoptotic rate as well. All values shown were mean ± SD of triplicate measurements and repeated three times with similar results.



Supplementary Figure S11. No significant effect on ROS production in the BCa cells after knockdown of *CENP-A*. (A) Flow cytometry analysis of ROS production in the *CENP-A-control-siRNA* (NC) and *CENP-A-target-specific-siRNA* (*CENP-A* KD) transfected BCa cells (UM-UC-3, T24 and EJ). **(B)** Statistical analysis revealed no significant (n. s.) changes of ROS production by reduction of *CENP-A*. All values shown were mean ± SD of triplicate measurements and repeated three times with similar results.



Supplementary Figure S12. Reduction of *CENP-A* exhibited no significant effect on apoptosis of the BCa cells. (A) Flow cytometry analysis for apoptosis in the *CENP-A-control-siRNA* (NC) and *CENP-A-target-specific-siRNA* (*CENP-A* KD) transfected BCa cells (UM-UC-3, T24 and EJ). **(B)** Statistical analysis revealed no significant (n. s.) changes of apoptotic rate by knockdown of *CENP-A*. **(C)** TUNEL staining for the apoptotic cells in the *CENP-A-control-siRNA* (NC) and *CENP-A-target-specific-siRNA* (*CENP-A* KD) transfected BCa cells (UM-UC-3, T24 and EJ). The scale bar for C is 40 μm. **(D)** Statistical analysis for TUNEL assay revealed no significant (n. s.) apoptotic rate as well. All values shown were mean ± SD of triplicate measurements and repeated three times with similar results.



Supplementary Figure S13. Schematic diagram of signaling mechanisms involved in the effects of *HJURP* on BCa. Knockdown of *HJURP* could upregulate PPARγ and downregulate SIRT1 partly through a CENP-A dependent manner. Then PPARγ and SIRT1 could regulate gene transcription and translation via a negative feedback loop by inducing cell cycle arrest at G0/G1 phase and ROS production. However, PPARγ-antagonist GW9662 and SIRT1 agonist could rescue this phenotype. As a consequence, knockdown of *HJURP* inhibited proliferation of BCa cells.

Supplementary Information 2

Supplementary Table S1. List of primers for qRT-PCR.

Gene	**Symbol**	**Forward primer (5'-3')**	**Reverse primer (5'-3')**	**Annealing Temperature (°C)**	**Length (bp)**
Holliday junction recognition protein	*HJURP*	5'-CACAAAGCCATCAAGCATCATC -3'	5'-TCAGAGCAGGGTATGAAGTTCT -3'	56	159
glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	5'-ACAACTTTGGTATCGTGGAAGG -3'	5'-GCCATCACGCCACAGTTTC -3'	56	101

Supplementary Table S2. List of *HJURP si-RNA* sequences.

Symbol	**Sense sequence (5’-3’)**	**Antisense sequence (5’-3’)**
HJURP siRNA-1 (*si-1*)	5'-CGACUCCAGUGCAACAUAUTT-3'	5'-AUAUGUUGCACUGGAGUCGTT-3'
HJURP siRNA-2 (*si-2*)	5'-GGAGAGAACACGUCUUACATT-3'	5'-UGUAAGACGUGUUCUCUCCTT-3'
HJURP siRNA-3 (*si-3*)	5'-AGUCGUAUCUCCAGAAAGATT-3'	5'-UCUUUCUGGAGAUACGACUTT-3'
Control siRNA (*NC*)	5'-UUCUCCGAACGUGUCAGGUTT-3'	5'-ACGUGACACGUUCGGAGAATT-3'

Supplementary Table S3. List of *CENP-A si-RNA* sequences.

Symbol	**Sense sequence (5'-3')**	**Antisense sequence (5'-3')**
CENP-A siRNA-1 (*si-1*)	5'-CUCUGUAACAGAGGUAAUATT-3'	5'-UAUUACCUCUGUUACAGAGTT-3'
CENP-A siRNA-2 (*si-2*)	5'-GUGACUAUUUGAGGAUUUUTT-3'	5'-AAAAUCCUCAAAUAGUCACTT-3'
CENP-A siRNA-3 (*si-3*)	5'-GGCUAAAGGAGAUCCGAAATT-3'	5'-UUUCGGAUCUCCUUUAGCCTT-3'
Control siRNA (*NC*)	5'-UUCUCCGAACGUGUCAGGUTT-3'	5'-ACGUGACACGUUCGGAGAATT-3'

Supplementary Table S4. List of primary antibodies.

Antigens	**Species antibodies raised in**	**Dilution (IF)**	**Dilution (WB)**	**Supplier**
Bax, human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #5023
Bcl-xl, human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #2764
Caspase-3, human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #9665
Caspase-7, human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #12827
Caspase-9, human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #9508
Catalase, human	Rabbit, monoclonal	1:200	1:2,000	Abcam, UK, Cat. #ab76024
CDK2, human	Rabbit, monoclonal	-	1:2,000	Abcam, UK, Cat. #ab32147
CDK4, human	Rabbit, monoclonal	-	1:2,000	Abcam, UK, Cat. #ab124821
CENP-A, human	Rabbit, monoclonal	-	1:2,000	Abcam, UK, Cat. #ab45694
Cleaved Caspase-3, human	Rabbit, monoclonal	-	1:500	Cell Signaling Technology, USA, Cat. #9664
Cleaved Caspase-7, human	Rabbit, monoclonal	-	1:500	Cell Signaling Technology, USA, Cat. #8438
Cleaved Caspase-9, human	Rabbit, monoclonal	-	1:500	Cell Signaling Technology, USA, Cat. #7237
Cyclin A1/2 (CCNA 1/2), human	Rabbit, monoclonal	-	1:2,000	Abcam, UK, Cat. #ab185619
Cyclin D1 (CCND1), human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #2978
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), human	Mouse, monoclonal	-	1:2,000	Santa Cruz Biotechnology Inc., USA, Cat. #sc-365062
GSK-3β, human	Rabbit, monoclonal	-	1:20,000	Cell Signaling Technology, USA, Cat. #12456
HJURP, human	Goat, polyclonal	1:100	-	Santa Cruz Biotechnology Inc., USA, Cat. #sc-168089
Hmox-1, human	Rabbit, monoclonal	-	1:1,000	Abcam, UK, Cat. #ab68477
Ki-67, human	Rabbit, polyclonal	1:200	-	Novus Biologicals, USA, Cat. #NBP2-19012
OCT4, human	Mouse, monoclonal	1:200	-	Novus Biologicals, USA, Cat. #NB110-90606

p38 MAPK, human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #8690
p44/42 MAPK (Erk1/2), rat	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #4695
p53 (acetyl K370) , human	Rabbit, monoclonal	1:500	1:1,000	Abcam, UK, Cat. #ab183544
Phospho-p38 (Thr180/Tyr182), human	Rabbit, monoclonal	-	1:1,000	Cell Signaling Technology, USA, Cat. #4511
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), human	Rabbit, monoclonal	-	1:1,000	Cell Signaling Technology, USA, Cat. #4370
Phospho-GSK-3β (Ser9) , human	Rabbit, monoclonal	-	1:20,000	Cell Signaling Technology, USA, Cat. #9475
Phospho-SAPK/JNK (Thr183/Tyr185), human	Rabbit, monoclonal	-	1:1,000	Cell Signaling Technology, USA, Cat. #4668
Phospho-SIRT1(Ser47), human	Rabbit, monoclonal	-	1:1000	Cell Signaling Technology, USA, Cat. #2314
PPARγ, human	Rabbit, monoclonal	1:500	1:1,000	Abcam, UK, Cat. #ab45036
SAPK/JNK, human	Rabbit, monoclonal	-	1:2,000	Cell Signaling Technology, USA, Cat. #9252
SIRT1, human	Rabbit, monoclonal	1:400	1:1,000	Cell Signaling Technology, USA, Cat. #9475
SOD2, human	Rabbit, monoclonal	-	1:1,000	Abcam, UK, Cat. #ab68155

Supplementary Table S5. List of secondary antibodies and counterstaining of nuclei.

Secondary detection system used	Host	Method	Dilution	Supplier
Anti-Mouse-IgG (H+L)-HRP	Goat	WB	1:10,000	Sungene Biotech, China, Cat. #LK2003
Anti-Rabbit-IgG (H+L)-HRP	Goat	WB	1:10,000	Sungene Biotech, China, Cat. #LK2001
Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate)	Goat	IF	1:50	Cell Signaling Technology, USA, Cat. #4412
Anti-mouse IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 555 Conjugate)	Goat	IF	1:50	Cell Signaling Technology, USA, Cat. #4408
Anti-goat IgG-FITC	Rabbit	IF	1:100	Boster Biological Technology, China, Cat. #BA1110
Anti-goat IgG-Cy3	Rabbit	IF	1:100	Boster Biological Technology, China, Cat. #BA1034
Hoechst 33342 nucleic acid staining (DAPI)	-	IF	1:750	Molecular Probes/Invitrogen, USA, Cat. #A11007

Supplementary Table S6. Top 30 significantly changed cell functions related with bladder cancer by mRNA microarray using bladder cancer tissues versus normal bladder tissues (ranking according to p-value).

<table>
<tr><th>GO ID</th><th>GO Name</th><th>Diff Gene Counts in GO</th><th>Gene Amount in GO</th><th>Enrichment Score</th><th>p-value</th><th>FDR</th><th>Gene Symbols</th><th>Rank</th></tr>
<tr><td>GO:0007165</td><td>signal transduction</td><td>111</td><td>1030</td><td>3.65632645</td><td>8.56E-32</td><td>2.90E-28</td><td>|PRKAR2B|PRKD1|PDE1C|IL1RAPL1|PPP3CB|DTNA|PPP2R5A|SPARCL1|MYO9A|IL8|TNFRSF21|PRKG1|PRKAA2|LYN|ADCY2|CDC42EP3|ARHGAP10|FPR3|CSF1R|FCGR2B|ARHGAP1|RTKN2|NR3C2|GNA14|CHRM3|ADCY9|TLE1|ITPKB|STAC|RARB|KITLG|IL1B|CD53|GKAP1|PTPLA|GULP1|PLAU|TNFRSF11B|SRGAP1|TLR3|CXCL1|ASB2|CCL18|STX2|PLXNC1|GNAL|EDNRA|RAP1A|PDE4D|PDE7B|SCGB1A1|SRGAP2|RASSF6|RASSF2|PDE5A|TYROBP|PDE9A|KIT|S100A9|PGR|NTS|ANXA1|AR|CLEC5A|IGF1|IPO8|PRKCB|CAP2|TNFSF8|ARHGAP11A|FYB|ARHGAP6|RYK|TNFAIP6|OPTN|NLRP3|IFNGR1|IL13RA2|AKT3|SPARC|ITPR1|CD4|IGFBP5|TLR2|EPS8|TLR6|CCL2|WISP1|ANGPT2|FPR1|C5AR1|RCAN1|IQGAP2|PLCB1|CXCL14|FCGR1A|RPS6KA6|PPP1R12A|P2RX1|HIF1A|CEACAM6|CXCL13|PLAUR|ITPR2|RACGAP1|CXCL5|WISP3|ARHGAP28|NAMPT|PECAM1|ARRB2|</td><td>1</td></tr>
<tr><td>GO:0007596</td><td>blood coagulation</td><td>73</td><td>465</td><td>5.32634284</td><td>2.62E-31</td><td>4.42E-28</td><td>|PDE5A|FCER1G|KIF4A|GATA6|LYN|KIF11|DGKE|SERPINE1|ITPR2|RAP1A|ANO6|PLEK|SPARC|COL1A2|PRKG1|JAM2|GNA14|PRKCB|TIMP1|SLC7A7|HIST2H3A|HIST1H3A|PRKAR2B|PLA2G4A|PLAU|SELL|FLNA|VCL|ATP1B3|RACGAP1|PLSCR4|LCP2|KIF15|MMP1|ARRB2|KIF23|DGKB|F13A1|PLAUR|AKAP1|JAM3|KIF18A|IGF1|SERPINB2|THBS1|SRGN|HBB|MRVI1|MERTK|OLR1|NRAS|TLN1|KIF2C|KCNMA1|CD36|TREM1|ITGAX|PDE9A|SLC8A1|TFPI2|ANGPT2|COL1A1|GNAI1|CD84|P2RX1|CENPE|PECAM1|PAPSS2|HABP4|PPBP|ITPR1|ACTN1|ITGAM|</td><td>2</td></tr>
</table>

GO:0006955	immune response	58	351	5.60634845	3.07E-26	3.47E-23	|FCGR2C|IGSF6|ZEB1|CD96|IGHG4|FCGR3B|IL1B|MS4A1|CCL2|IL1R1|SBSPON|IL8|CXCL1|GPR65|CTSC|TGFBR3|ARHGDIB|CCR1|CXCL2|HLA-DRA|CD28|C1QC|BMPR1A|OAS2|PTGER4|PRG4|THBS1|CR2|AQP9|CXCL13|IL6|FCGR3A|IGKC|CXCL5|FCGR2B|FCGR1B|CLEC4E|CD4|FCGR1A|HLA-DQB1|CCL18|IL2RA|CD86|TLR6|HLA-DPA1|CTSS|CXCL14|C5AR1|HLA-DMA|GBP2|CX3CL1|TNFSF8|IGHM|FYB|SLC11A1|LCP2|IL18|TLR2|	3
GO:0051301	cell division	53	295	6.09555232	7.41E-26	6.26E-23	|CDK1|CCNB1|CASC5|NCAPG2|SGOL1|SKA3|CDCA2|BUB1|CCNG1|KIF11|ANAPC16|CDCA8|NCAPG|CDC25C|PELO|NCAPH|GNAI1|NUF2|CDC6|KIF20B|CKS2|SPG20|KIF2C|NUMA1|KNTC1|CENPE|SKA1|RCC2|UBE2C|KLHL42|KNSTRN|AURKA|HELLS|CCNA2|CCNB2|MAD2L1|CDC7|PARD3B|CCND1|OIP5|CDC20|BORA|CENPF|LRRCC1|SMC4|PTTG1|MIS18BP1|CCND2|CCNE2|NDC80|NEK2|ZWILCH|TPX2|	4
GO:0007155	cell adhesion	63	454	4.70808079	3.91E-24	2.64E-21	|CNTN1|TGFBI|ITGB8|NLGN4Y|THEMIS2|NRXN1|COMP|FPR2|ADAM12|B4GALT1|MFAP4|DPT|TGFB1I1|CD96|THBS1|FLRT3|EGFL6|TNFAIP6|WISP1|ADAM17|PLXNC1|TLN1|CX3CL1|GNE|VCL|SELE|MPDZ|ITGBL1|DPP4|ITGAM|EPHA3|COL12A1|KITLG|CNTN3|KIAA1462|STAB1|DST|POSTN|NLGN1|CDH3|COL19A1|EMB|FLOT2|CCL2|ITGAX|OLFM4|THBS2|VCAN|DSC2|CCR1|NEGR1|CD36|CPXM1|NRP2|CD4|SELL|VCAM1|TNC|SPP1|PGM5|LPP|PECAM1|NCAM1|	5
GO:0000278	mitotic cell cycle	53	363	4.95368577	1.72E-21	9.71E-19	|PLK1|CENPN|ESCO2|PTTG1|TOP2A|NEK2|CASC5|MCM10|CDC6|CDC7|PRKAR2B|CENPK|CDK1|PLK4|PCM1|CDC20|OPTN|TYMS|CENPI|KIF23|ZWILCH|UBE2C|CENPE|CCNA2|CCND1|AURKB|NUMA1|NCAPH|KNTC1|KIF2C|CCNB1|PPP1R12A|MAD2L1|MLF1IP|CDCA8|POLE2|SMC4|BUB1|SGOL1|MYBL2|CENPF|AURKA|KIF18A|RRM2|KIF20A|RCC2|CCNE2|NUF2|SKA1|NCAPG|CCNB2|NDC80|CDC25C|	6
GO:0000236	mitotic prometaphase	28	100	9.49986079	1.37E-19	6.62E-17	|ZWILCH|CENPK|CENPN|CCNB1|MAD2L1|CDC20|SMC4|KIF2C|BUB1|PLK1|AURKB|CENPF|RCC2|NCAPH|SGOL1|CENPE|CENPI|SKA1|CDCA8|NDC80|MLF1IP|KNTC1|KIF18A|CDK1|CASC5|NUF2|NCAPG|CCNB2|	7

GO:0044281	small molecule metabolic process	104	1363	2.58878923	2.81E-18	1.19E-15	|GK|NT5E|ADCY2|SLC23A2|ACACB|HEXA|CYP1B1|ALDH6A1|ADH1B|PDK4|PYGM|CH25H|KMO|ITPKB|SLC44A5|CYP3A5|UST|KYNU|RRM2|CYP4F3|ITPR2|TNFRSF21|ACER2|ACACA|PLBD1|GLA|RAP1A|CTGF|TDO2|INPP5A|NNMT|CHRM3|ADH1C|AMY2A|NQO1|HBB|HPSE2|NAMPT|CYP24A1|PLA2G2A|HMMR|BGN|EPHX2|VCAN|CYP2J2|PLCB4|CYP4B1|PRELP|BHMT|PLD3|IPMK|FBP1|PTGS1|LPL|ADCY9|GALC|PTGIS|LPA|PRKAR2B|ACSL1|SLC2A3|MAOB|PLCB1|PRKAA2|BCAT1|NPAS2|PCCA|PCBD1|AASS|GSTA4|PPAP2B|PLA2G4A|TBL1X|ACAT1|B4GALT4|SLC25A4|HMOX1|HPGD|PGD|CKB|PAPSS2|SAT1|ACADSB|PYGB|UCP2|SQLE|TYMS|BRIP1|SLC22A3|MTAP|CERS3|INS|SRD5A2|CD36|B4GALT1|HBA1|CSGALNACT1|ITPR1|SGPP1|ADHFE1|LGMN|DHCR24|MAOA|PRKD1|	8
GO:0030198	extracellular matrix organization	37	210	5.97780356	4.40E-18	1.65E-15	|COL1A2|CSGALNACT1|MMP12|TIMP1|COL3A1|EGFL6|FOXF2|ITGA8|COL19A1|MFAP4|MMP13|ITGB8|POSTN|COL4A1|MMP16|MMP1|COL11A1|COL5A2|B4GALT1|ADAM17|SPINK5|FBN1|COL4A5|COL10A1|TNFRSF11B|NDNF|COL12A1|MMP9|DST|MMP19|TGFBI|LOX|COL21A1|COL1A1|OLFML2B|FOXF1|MFAP5|	9
GO:0045087	innate immune response	60	554	3.67452068	1.23E-17	4.02E-15	|TYROBP|CD36|CD14|ITPR2|TLR6|LCP2|TXNIP|NRAS|LY86|FCGR1A|ADCY2|IGKC|IGLC7|C1QC|BTK|TLR2|DMBT1|EREG|C2|CTSS|PGLYRP3|KL|CD28|LY96|PRKAR2B|FCGR3A|C7|IGHG4|CSF1R|C1QB|PDE1C|TLR3|NCF2|CDK1|LGMN|NLRP3|CD209|KIT|ITPR1|CLEC7A|RPS6KA5|GAB1|FCGR2A|CD86|CR2|S100A9|CR1|KITLG|CD4|PELI2|BTC|CD180|LYN|ITGAM|ADCY9|CLEC5A|CYBB|TREM1|FGF10|PRKD1|	10
GO:0006954	inflammatory response	43	295	4.94544811	1.31E-17	4.02E-15	|SELE|CXCR4|THEMIS2|TLR3|S100A9|KIT|IL8|LYZ|CXCL2|SLC11A1|IL10|CCL18|CYBB|CCR1|PRKD1|STAB1|IL1B|CCL2|TNFAIP6|IL6|C3AR1|EPHX2|CD14|CD180|RPS6KA5|CSF1R|SPP1|CCL3|IL18|TLR6|CXCL1|OLR1|LY96|PTX3|ANXA1|THBS1|FPR2|LY86|NLRP3|TLR2|CHI3L1|C5AR1|CLEC7A|	11
GO:0000087	M phase of mitotic cell cycle	35	198	5.99738686	3.26E-17	9.19E-15	|CCNB1|MAD2L1|NCAPH|CENPE|KIF18A|CDC25C|CCNB2|CASC5|NUF2|SGOL1|AURKB|CENPN|CENPK|CENPF|DLGAP5|SKA1|NCAPG|PTTG1|PLK1|KIF23|CDCA8|CDC20|MLF1IP|BUB1|CENPI|KIF20A|KNTC1|CDK1|KIF2C|SMC4|NDC80|RCC2|KIF20B|ZWILCH|UBE2C|	12

GO:0008283	cell proliferation	45	336	4.54393852	6.20E-17	1.61E-14	|E2F8|CSGALNACT1|EMP1|ZEB1|AREG|EMP2|CENPF|STIL|USP28|ZFP36L2|GAB1|KRT16|PELO|TPX2|KIF15|PRKD1|PRG4|BCAT1|MKI67|IL2RA|CSF1R|AURKB|ZMYND11|ELF5|CKS2|MELK|LY86|SRGAP2|AR|PLK1|KIF2C|TGFBI|CDC25C|KITLG|CXCL1|TNFSF8|ILK|BUB1|DAB2|DLGAP5|EPS8|CHRM3|TBX20|YAP1|EDNRA|	13
GO:0030168	platelet activation	35	204	5.82099313	8.65E-17	2.09E-14	|TIMP1|SRGN|DGKB|ITPR2|LYN|COL1A2|MERTK|LCP2|GNA14|IL6|PLEK|SPARC|TLN1|COL1A1|COL3A1|F13A1|PECAM1|PPBP|RAP1A|IGF1|ITPR1|GNAI1|SERPINE1|PRKCB|P2RX1|FCER1G|ARRB2|THBS1|FLNA|HABP4|DGKE|VCL|PLA2G4A|CD36|ACTN1|	14
GO:0007067	mitosis	32	182	5.96537569	9.16E-16	2.06E-13	|LRRCC1|KIF11|KIF20B|CDCA2|KIF15|NDC80|NEK2|AURKA|MIS18BP1|PLK1|CDC6|ASPM|CIT|NCAPH|TPX2|OIP5|HELLS|CEP55|CCNG1|NUF2|BORA|ANAPC16|CDC25C|NCAPG2|SKA3|CCNA2|ANLN|SKA1|PPP1R12A|KIF2C|PBK|KLHL42|	15
GO:0008284	positive regulation of cell proliferation	48	411	3.96240283	1.36E-15	2.88E-13	|GREM1|NCCRP1|TNC|MYOCD|FOXM1|CDC20|LYN|TTK|ADAM17|INS|ID4|HAS2|AR|KIT|DPP4|LEP|ACER2|BAMBI|EREG|CDC7|TSPYL5|LIFR|NRAS|IL6|GLP2R|CD86|HILPDA|ILK|CSF1R|SLC25A27|HCLS1|CXCL5|IGF1|NAMPT|SFRP2|RTKN2|BTC|AKR1C2|PTHLH|YAP1|PBX1|TBX3|TIMP1|EDNRA|SIRT1|KRT6A|ST8SIA1|CTGF|	16
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	63	708	3.01902356	2.30E-14	4.57E-12	|SLC11A1|YAP1|FOXF2|SERPINE1|E2F8|NFIB|HCLS1|MYOCD|CDH13|TLR2|IL33|IL10|IL6|RARB|KLF12|FOXF1|NPAS2|IGF1|TOP2A|TBX20|PYGO1|PBX1|ID4|INHBA|GATA6|CENPK|AR|TLR3|SMARCA2|MEIS2|ARNTL2|TBX5|FGF10|GREM1|SFRP2|CD28|ZNF462|HIF1A|SOX5|FOXM1|LDB1|NAMPT|GLI3|KAT2B|E2F7|RPS6KA5|TEAD1|IL1B|PPP1R12A|BMP5|EGR1|HOXA13|ZEB1|TNFSF8|EYA1|SIRT1|CKAP2|RLF|TBL1X|PKD2|NFIX|PRKD1|PITX2|	17
GO:0006915	apoptotic process	60	654	3.11266736	2.58E-14	4.85E-12	|TJP2|DSG3|PRKD1|SULF1|ACTC1|IL1B|GREM1|ARHGEF9|DHCR24|ARHGAP10|SEMA3A|ARHGEF12|CASP14|KANK2|CDK1|PRUNE2|IL2RA|GPR65|RNF144B|GULP1|CXCR4|DAB2|BUB1|C5AR1|ID1|PHLDA1|EGLN3|DSG1|TOX3|BCL2L11|MELK|ECT2|UNC5C|PRKCB|AKTIP|PEG3|CLSPN|LMNB1|TJP1|MST4|SGK1|CD14|GADD45B|PAK6|STK17B|COMP|TPX2|SATB1|MAPT|GATA6|LY86|RASSF6|FGD4|BCL2A1|HIPK3|TNFRSF11B|PDCL3|CKAP2|SGPP1|EPHA3|	18

<table>
<tr><td>GO:0006936</td><td>muscle contraction</td><td>22</td><td>95</td><td>7.85702772</td><td>9.46E-14</td><td>1.68E-11</td><td>|GALR2|ACTG2|TACR2|ITGA1|MYL9|LMOD1|SLC8A1|VCL|MYH11|TLN1|CACNA1H|SORBS1|TPM1|ACTA2|TBX20|SCN7A|KCNE1|TPM2|MYOM1|DES|CALD1|SLMAP|</td><td>19</td></tr>
<tr><td>GO:0035556</td><td>intracellular signal transduction</td><td>36</td><td>284</td><td>4.30074181</td><td>4.89E-13</td><td>8.26E-11</td><td>|DGKE|TNS1|CTGF|PRKAR2B|ASB2|ROCK2|ADCY9|STAC|PREX2|CDC42BPA|CXCL1|ASB5|ARHGEF12|CD209|DEPDC1|INADL|GRIP1|HCLS1|MYO9A|PLCD4|TYROBP|PLCB1|PRKCB|PLCB4|ECT2|IL8|MTMR8|ADCY2|DGKB|TREM1|PLCL1|PRKD1|BTK|IGFBP5|LYN|CIT|</td><td>20</td></tr>
<tr><td>GO:0019221</td><td>cytokine-mediated signaling pathway</td><td>31</td><td>216</td><td>4.86930695</td><td>7.96E-13</td><td>1.28E-10</td><td>|FCGR1B|EIF4E3|MT2A|EGR1|CAMK2G|CSF1R|KIT|HLA-DRB4|OAS2|IRAK3|HLA-DQB1|EREG|IL1B|VCAM1|IFI30|KPNA5|PLP2|CX3CL1|HLA-DPA1|IFNGR1|LIFR|KPNA2|NCAM1|HLA-DRA|CCR1|CCL2|IL6|GBP2|NEDD4|IL13RA2|FCGR1A|</td><td>21</td></tr>
<tr><td>GO:0006334</td><td>nucleosome assembly</td><td>22</td><td>108</td><td>6.91127438</td><td>1.55E-12</td><td>2.39E-10</td><td>|HIST1H2BD|CASC5|OIP5|HIST1H2BM|HIST1H2BK|TSPYL5|CENPN|H2AFX|CENPK|HIST1H2BJ|KAT6B|MIS18BP1|HIST1H3A|HIST2H3A|MLF1IP|HIST1H2BO|HIST1H2AB|HIST1H2BB|HIST1H2AG|HJURP|HIST1H2BH|CENPI|</td><td>22</td></tr>
<tr><td>GO:0007411</td><td>axon guidance</td><td>37</td><td>317</td><td>3.96005914</td><td>2.89E-12</td><td>4.25E-10</td><td>|CDK1|GLI3|KIF4A|UNC5C|MYL9|NRXN1|ROCK2|ARHGEF12|CNTN1|RRAS|MATN2|RPS6KA6|TRPC1|SEMA3A|CACNA1H|SRGAP2|NRP2|PLXNC1|NRAS|COL3A1|TLN1|SEMA3E|COL5A2|RYK|COL4A5|CACNB2|TYROBP|CAP2|PTPRC|ST8SIA4|MYH11|COL4A1|SRGAP1|NCAM1|ITGA1|RPS6KA5|SLIT2|</td><td>23</td></tr>
<tr><td>GO:0007267</td><td>cell-cell signaling</td><td>32</td><td>242</td><td>4.48635693</td><td>3.25E-12</td><td>4.59E-10</td><td>|WISP1|INHBA|PGR|IL18|CEACAM6|AR|STAB1|TBX5|CCL18|DLGAP5|CCR1|AREG|S100A9|TNFSF8|INS|TNFAIP6|CD86|MERTK|CCL3|WISP3|PTHLH|GREM1|CXCL14|CXCL5|IL1B|EREG|IL10|GJB2|NAMPT|CXCL13|SRD5A2|SFRP2|</td><td>24</td></tr>
<tr><td>GO:0001525</td><td>angiogenesis</td><td>29</td><td>201</td><td>4.89509529</td><td>3.95E-12</td><td>5.34E-10</td><td>|EREG|SERPINE1|CYP1B1|HIF1A|MMP19|ANG|NRXN1|NRP2|ANPEP|CCL2|TGFBI|VEZF1|ID1|HMOX1|IL18|COL4A1|PRKD1|JAM3|THSD7A|SIRT1|CLIC4|THY1|IL8|FGF10|ANGPT2|CTGF|CALCRL|SAT1|MEIS1|</td><td>25</td></tr>
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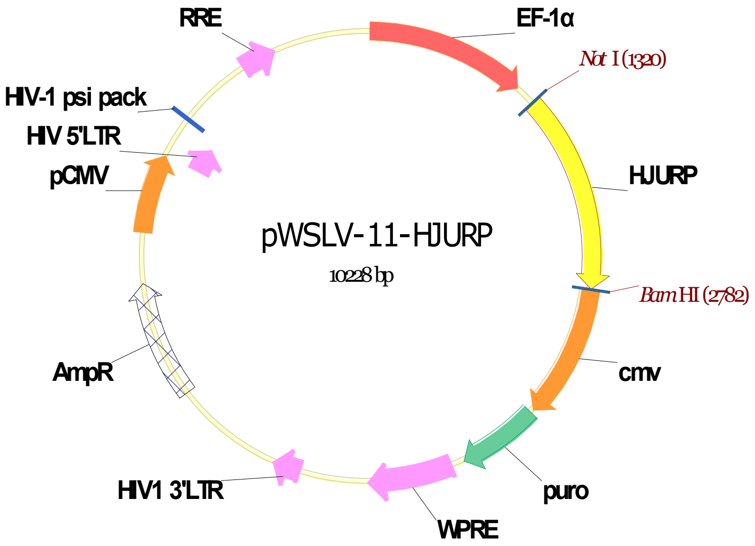
<tr><td>GO:0000122</td><td>negative regulation of transcription from RNA polymerase II promoter</td><td>48</td><td>509</td><td>3.19950405</td><td>4.32E-12</td><td>5.61E-10</td><td>|PITX2|KLF12|AURKB|NRARP|SIRT1|TBX20|ID4|TAF9B|LEP|PLK1|SATB1|TBL1X|ID1|ZEB1|KANK2|PTCH1|EGR1|MEIS2|CREBRF|HCFC2|CD36|FOXM1|GLI3|E2F8|NEDD4L|NFIX|NFIB|AES|PRDM1|ZMYND11|ZHX2|ZBTB18|MYOCD|EZH2|E2F7|SMARCA2|SALL2|RARB|CRY1|SCGB1A1|PRRX1|GATA6|TBX3|EZH1|FOXF1|TXNIP|NR1D1|TCEAL1|</td><td>26</td></tr>
<tr><td>GO:0019886</td><td>antigen processing and presentation of exogenous peptide antigen via MHC class II</td><td>20</td><td>92</td><td>7.37566831</td><td>4.72E-12</td><td>5.91E-10</td><td>|KIF23|KIF4A|KIF2C|KIF15|DYNC2H1|FCER1G|HLA-DMA|KIF18A|HLA-DPA1|HLA-DRB4|DYNC1I1|HLA-DRA|RACGAP1|LGMN|CENPE|CTSS|KIF11|HLA-DQB1|IFI30|OSBPL1A|</td><td>27</td></tr>
<tr><td>GO:0000090</td><td>mitotic anaphase</td><td>26</td><td>164</td><td>5.37884104</td><td>6.05E-12</td><td>7.18E-10</td><td>|SGOL1|SKA1|KIF2C|CDC20|AURKB|ZWILCH|KIF18A|RCC2|NUF2|KNTC1|CENPF|NDC80|PTTG1|CENPK|CENPE|PLK1|MLF1IP|MAD2L1|BUB1|CENPN|NUMA1|CENPI|UBE2C|KNSTRN|CDCA8|CASC5|</td><td>28</td></tr>
<tr><td>GO:0001501</td><td>skeletal system development</td><td>23</td><td>127</td><td>6.14445439</td><td>6.30E-12</td><td>7.18E-10</td><td>|COL1A1|COL1A2|BMP5|ADAMTS4|HOXA11|COL10A1|COL5A2|PTHLH|POSTN|TBX3|AES|COMP|COL12A1|PRELP|HOXA13|COL3A1|PAPSS2|MMP9|FBN1|IGF1|TNFRSF11B|HEXA|COL19A1|</td><td>29</td></tr>
</table>

Supplementary Information 3

Supplementary Sequences S1. Sequences of *pWSLV-11-HJURP* plasmid.

5’-NNNNNNNGNTCNTTGANTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAGCATGCGATGACTGATCATGACCCTCGAGGTCGACGGTATCGATAAGCTCGCTTCACGAGATTCCAGCAGGTCGAGGGACCTAATAACTTCGTATAGCATACATTATACGAAGTTATATTAAGGGTTCCAAGCTTAAGCGGCCGCGCCACCATGCTGCACTCCATGAGCCGGCTGTTGAGCACAAAGCCATCAAGCATCATCTCCACCAAAACGTTCATCATGCAAAACTGGAACTGCAGGAGGAGGCACAGATATAAGAGCAGGATGAACAAAACATATTGCAAAGGAGCCAGACGTTCTCAGAGGAGCTCCAAGGAGAACTTCATACCCTGCTCTGAGCCTGTGAAAGGGACAGGGGCATTAAGAGATTGCAAGAACGTATTAGATGTTTCTTGCCGTAAGACAGGTTTAAAATTGGAAAAAGCTTTTCTTGAAGTCAACAGACCCCAAATCCATAAGTTAGATCCAAGTTGGAAGGAGCGCAAAGTGACACCCTCGAAGTATTCTTCCTTGATTTACTTCGACTCCAGTGCAACATATAATCTTGATGAGGAAAATAGATTTAGGACATTAAAATGGTTAATTTCTCCTGTAAAAATAGTTTCCAGACCAACAATACGACAGGGCCATGGAGAGAACCGTCAGAGGGAGATTGAAATCCGATTTGATCAGCTTCATCGGGAATATTGCCTGAGTCCCAGGAACCAGCCTCGCCGGATGTGCCTCCCGGACTCCTGGGCCATGAACATGTACAGAGGGGGTCCTGCGAGTCCTGGTGGCCTTCAGGGCTTAGAAACCCGCAGGCTGAGTTTACCTTCCAGCAAAGCAAAAGCAAAAAGTTTAAGTGAGGCTTTTGAAAACCTAGGCAAAAGATCTCTGGAAGCAGGTAGGTGCCTGCCCAAGAGCGATTCATCTTCATCACTTCCAAAGACCAACCCCACACACAGCGCAACTCGCCCGCAGCAGACATCTGACCTTCACGTTCAGGGAAATAGTTCTGGAATATTTAGAAAGTCAGTGTCACCCAGCAAAACTCTTTCAGTCCCAGATAAAGAAGTGCCAGGCCACGGAAGGAATCGTTACGATGAAATTAAAGAAGAATTTGACAAGCTTCATCAAAAGTATTGCCTCAAATCTCCTGGGCAGATGACAGTGCCTTTATGTATTGGAGTGTCTACAGATAAAGCAAGTATGGAAGTTCGATATCAAACAGAAGGCTTCTTAGGAAAATTAAATCCAGACCCTCACTTCCAGGGTTTCCAGAAGTTGCCATCATCACCCCTGGGGTGCAGAAAAAGTCTACTGGGCTCAACTGCAATTGAGGCTCCTTCATCTACATGTGTTGCTCGTGCCATCACGAGGGATGGCACGAGGGACCATCAGTTCCCTGCAAAAAGACCCAGGCTATCAGAACCCCAGGGCTCCGGACGCCAGGGCAATTCCCTGGGTGCCTCAGATGGGGTGGACAACACCGTCAGACCGGGAGACCAGGGCAGCTCTTCACAGCCCAACTCAGAAGAGAGAGGAGAGAACACGTCTTACAGGATGGAAGAGAAAAGTGATTTCATGCTAGAAAAATTGGAAACTAAAAGTGTGTAGGGATCCATATATTTCGACCATAGCCAATTCAATATGGCGTATATGGACTCATGCCAATTCAATATGGTGGATCTGGACCTGTGCCAATTCAATATGGCGTATATGGACTCGTGCCAATTCAATATGGTGGATCTGGACCCCAGCCAATTCAATATGGCGGATTGGCCNACAATTGCCCAATCCNATTATGG-3’



Map of the *pWSLV-11-HJURP* plasmid.