Supplemental information

The UCHL5 inhibitor b-AP15 overcomes

cisplatin resistance via suppression

of cancer stemness in urothelial carcinoma

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Table S1. List of primer sequences used for real-time quantitative PCR analysis.

GAPDH Forward	5'-GCATTGCCCTCAACGAC-3'
GAPDH Reverse	5'-GTCTCTCTCTTCCTCTTGTGC-3'
MYC Forward	5'-TGTCCGTCCAAGCAGAGGAGCA-3'
MYC Reverse	5'-TCAGCCAAGGTTGTGAGGTTGC-3'

Table S2. CI for experimental values in BFTC905 cells

b-AP15(nM)	Cisplatin (nM)	Fraction Affected (Fa)	Combination Index (CI)
62.5	625	0.18	0.750
125	1250	0.32	0.725
250	2500	0.49	0.749
500	5000	0.65	0.827
750	7500	0.68	1.099

Table S3. CI for experimental values in T24 cells

b-AP15(nM)	Cisplatin (nM)	Fraction Affected (Fa)	Combination Index (CI)
100	1500	0.28	0.669
150	2250	0.40	0.803
200	3000	0.62	0.755
250	3750	0.73	0.783
300	4500	0.75	0.905

Table S4. CI for experimental values in T24/R cells

b-AP15(nM)	Cisplatin (nM)	Fraction Affected (Fa)	Combination Index (CI)
250	3750	0.24	0.748
500	7500	0.41	0.646
750	11250	0.46	0.781
1000	15000	0.51	0.843
1250	18750	0.53	0.968

Table S5. CI for experimental values in RT4 cells

| b-AP15(nM)	Cisplatin (nM)	Fraction Affected (Fa)	Combination Index (CI)
100	1500	0.28	0.669
150	2250	0.40	0.803
200	3000	0.62	0.755
250	3750	0.73	0.783
300	4500	0.75	0.905

Table S6.

RNA sequencing data from DMSO or b-AP15-treated T24/R cells was analyzed via gene set enrichment analysis (GSEA). Target genes enrichment scores of the GSEA FEVR_CTNNB1_TARGETS_DN were listed in this heatmap. This table is attached in "Supplemental Spreadsheet".
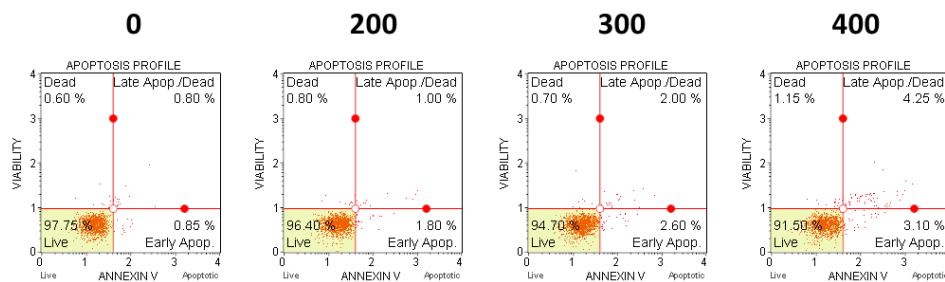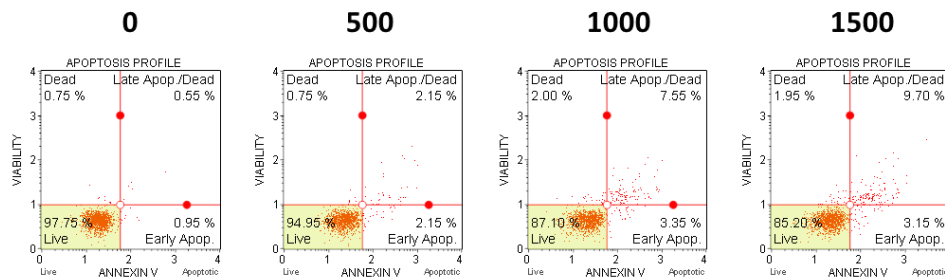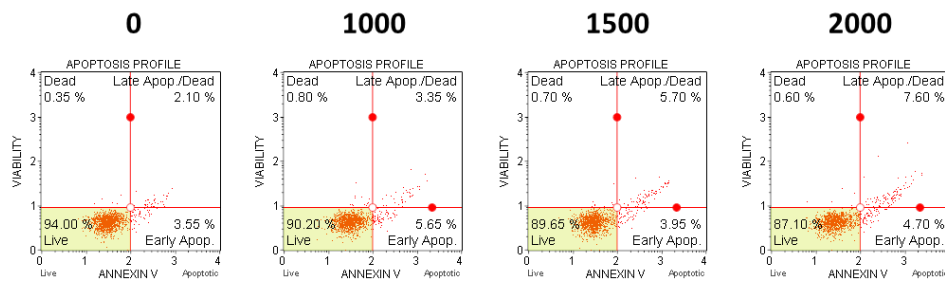
Figure S1
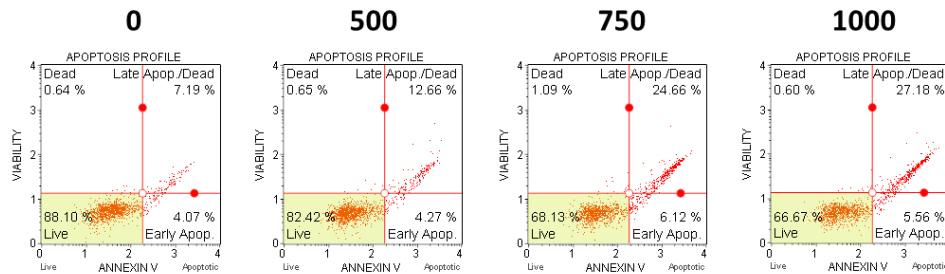
Figure S1.
The UC cells were treated with b-AP15 at indicated concentrations for 24 hours and the apoptotic cell population was analyzed using flow cytometry as described in Methods.

Figure S2

YU_MYC_TARGETS_UP Heatmap

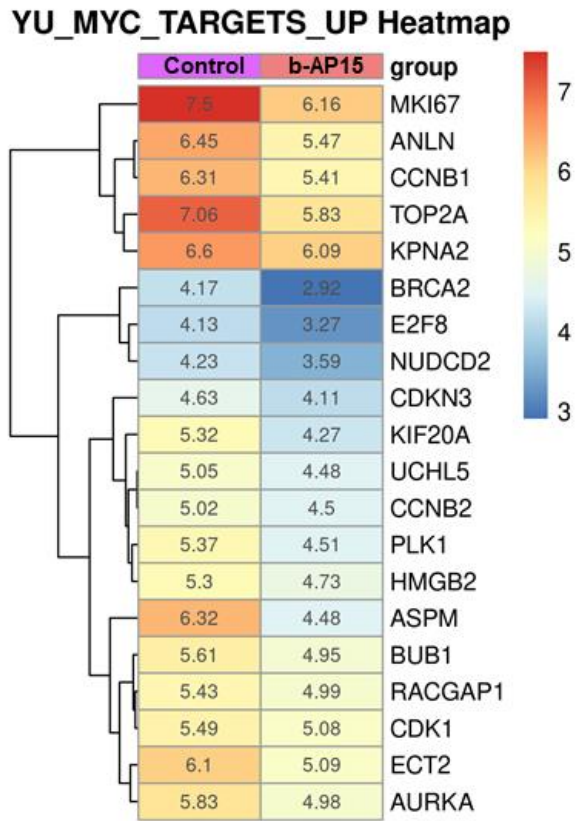|	Control	b-AP15	group
	7.5	6.16	MKI67
	6.45	5.47	ANLN
	6.31	5.41	CCNB1
	7.06	5.83	TOP2A
	6.6	6.09	KPNA2
	4.17	2.92	BRCA2
	4.13	3.27	E2F8
	4.23	3.59	NUDCD2
	4.63	4.11	CDKN3
	5.32	4.27	KIF20A
	5.05	4.48	UCHL5
	5.02	4.5	CCNB2
	5.37	4.51	PLK1
	5.3	4.73	HMGB2
	6.32	4.48	ASPM
	5.61	4.95	BUB1
	5.43	4.99	RACGAP1
	5.49	5.08	CDK1
	6.1	5.09	ECT2
	5.83	4.98	AURKA

Figure S2.

RNA sequencing data from DMSO or b-AP15-treated T24/R cells was analyzed via gene set enrichment analysis (GSEA). Target genes enrichment scores of the GSEA YU_MYC_TARGETS_UP were listed in this heatmap.
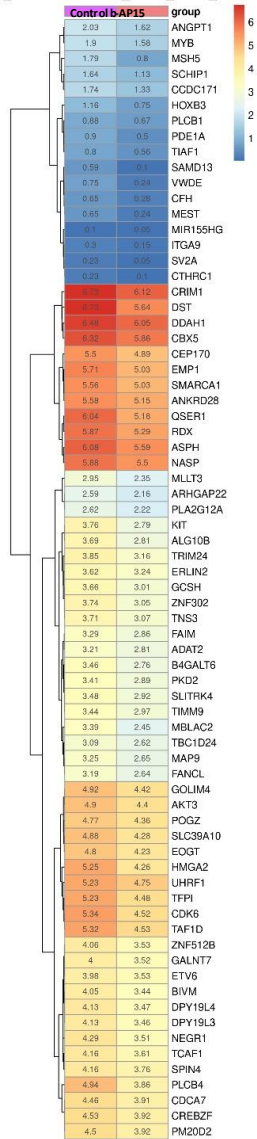
Figure S3
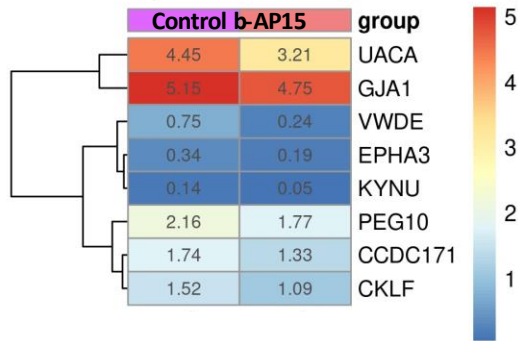
Figure S3.

RNA sequencing data from DMSO or b-AP15-treated T24/R cells was analyzed via gene set enrichment analysis (GSEA). Target genes enrichment scores of the GSEA LI_CISPLATIN_RESISTANCE_UP were listed in this heatmap.

Figure S4

Figure S4.

RNA sequencing data from DMSO or b-AP15-treated T24/R cells was analyzed via gene set enrichment analysis (GSEA). Target genes enrichment scores of the GSEA JAATINEN_HEMATOPOIETIC_STAM_CELL_UP were listed in this heatmap.
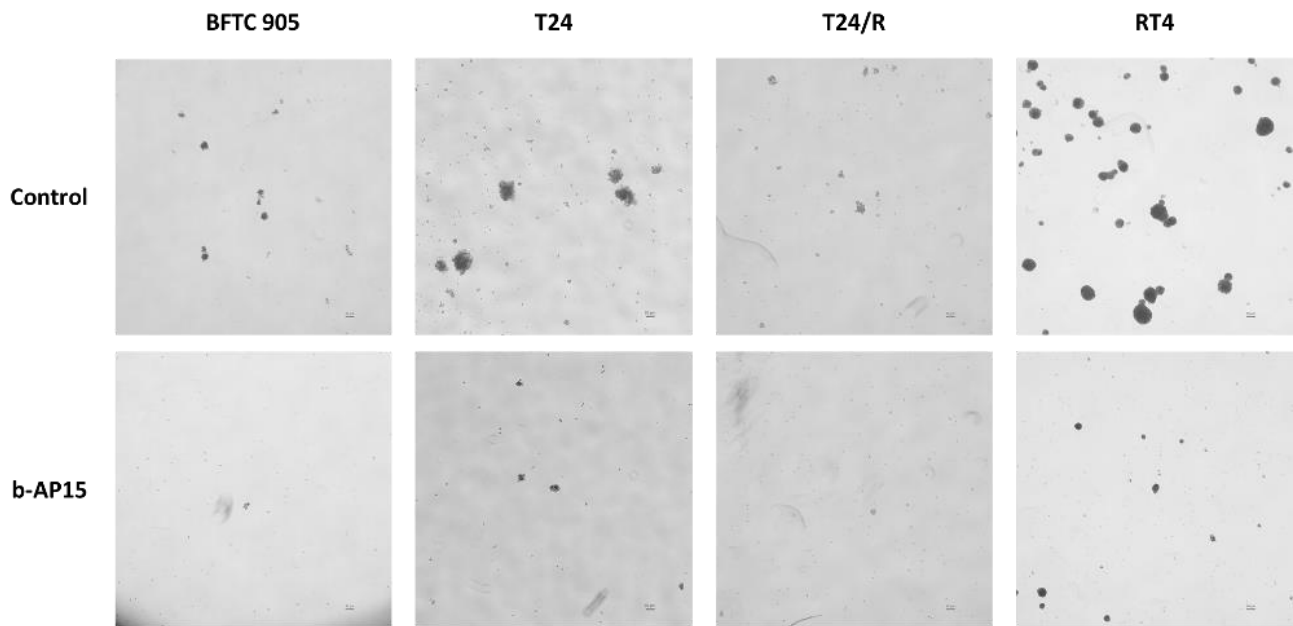
Figure S5

Figure S5.

The cells were subjected to tumorsphere cultivation assay within the treatment of DMSO (control) or low-dose b-AP15 (50 nM) for 14 days. The images were digitized at 40× magnification. Scale bars, 50 mm.
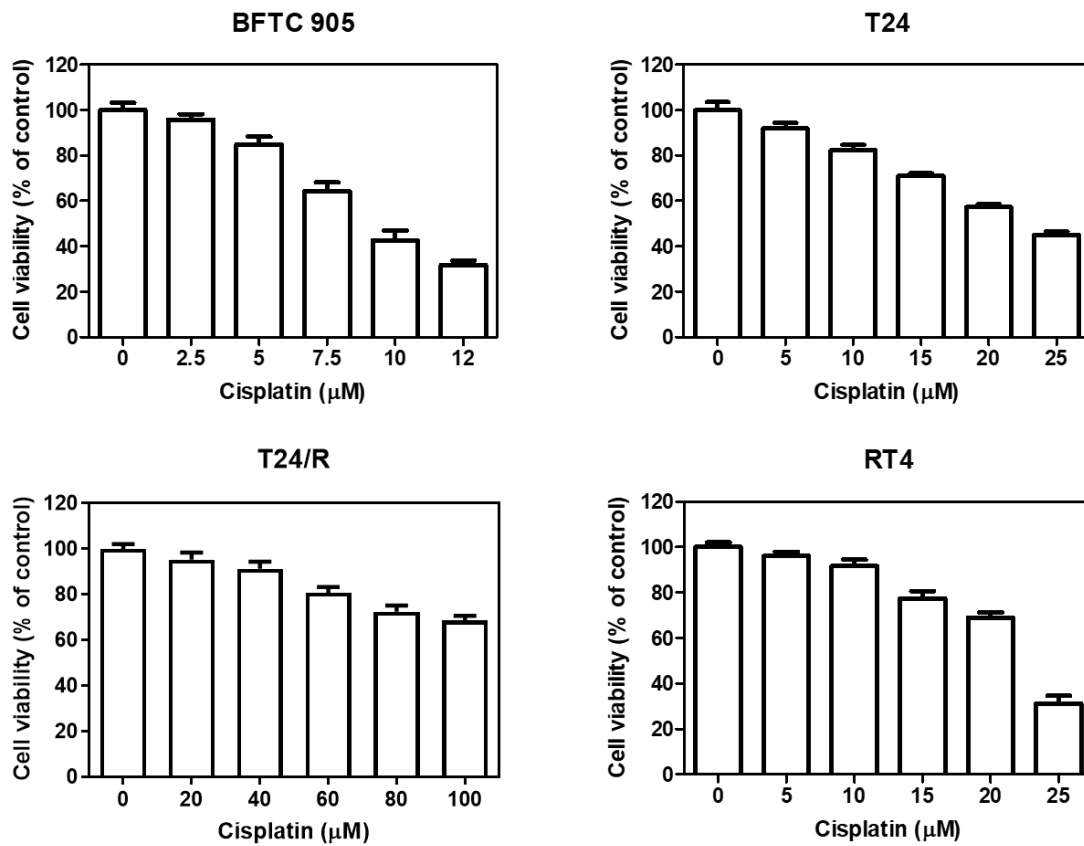
Figure S6

Figure S6.

The cells were treated with indicated concentrations of cisplatin for 24 hours, followed by the MTT assay to analyze cell viability. The following experiments which combined b-AP15 and cisplatin to treat the UC cells were based on these results.