

Targeting XBP1-mediated β -catenin expression associated with bladder cancer with newly synthetic Oridonin analogues

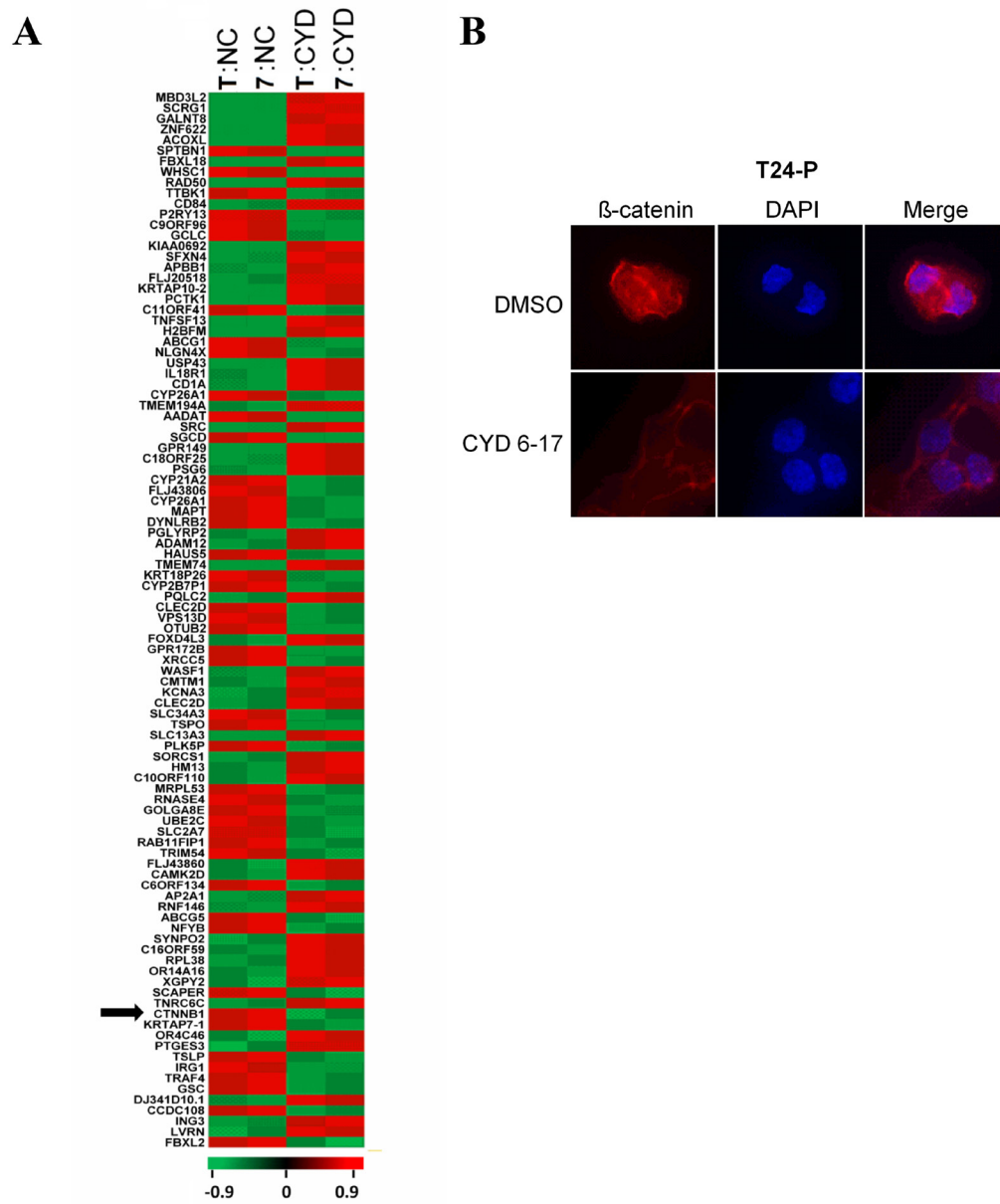
SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1: Primers for qRT-PCR, CTNNB1 promoter cloning and ChIP assay

Primers for qRT-PCR		
Gene	Forward	Reverse
18S	GGAATTGACGGAAGGGCACCACC	GTGCAGCCCCGGACATCTAAGG
CTNNB1	ACTACTGTGGACCACAAGCAGAG	AGTCCCAAGGAGACCTTCCATC
XBP1	GGAGTTAAGACAGCGCTTGG	ACTGGGTCCAAGTTGTCCAG
Primers for CTNNB1 promoter cloning		
Forward	GCTGCGATGCGGTACCGAACGAACCTTCTTTACAATACTGGCATAT	
Reverse	GCGACTCGATCTCGAGACGCTGCTGCCACAGACCGAGAGGCTTAAAAT	
Primers for ChIP assay		
Set	Forward	Reverse
Control	AAGCCTCTGTTTTAGCGAAGG	ACCCCGGGTTTGACATAAAT
P1	CCCGATGCAGACCACAG	CGCAGAAGCCGCTGTAT
P2	GCTCAGACGGCAGCAGAC	GACCGAGAGGCTTAAAATGG

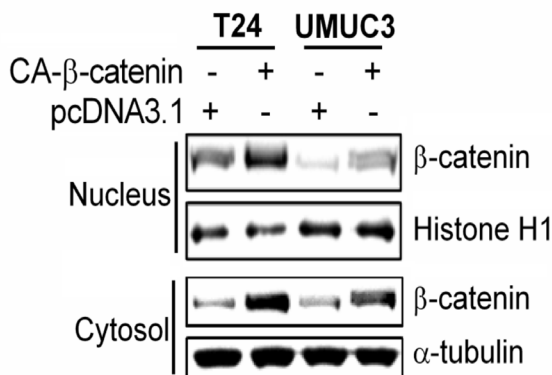
Supplementary Table S2: IC₅₀ of TCC-SUP, T24, and UMUC3 for various chemotherapeutics (www.cancerrxgene.org)

Cell line	IC50 (μM)					
	Cisplatin	Doxorubicin	Vinblastine	Methotrexate	Mitomycin C	Gemcitabine
TCC-SUP	5.5616	0.2455	0.0051	13.9548	0.2458	2.0763
T24	7.0357	0.0860	0.0079	12.7190	0.1018	0.0162
UMUC3	8.4988	1.2308	0.0236	8.5696	0.4817	1.1679

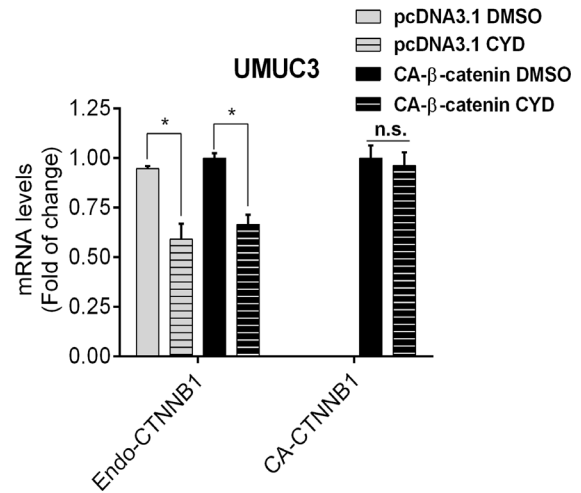


Supplementary Figure S1: **A.** Heat map of the gene expression profile regulated by CYD 6-17 treatment in T24-B and 786-O. Black arrow indicates CTNNB1. T: T24-B; 7: 786-O. NC: negative control; CYD: CYD 6-17 treated. Scale bar shown for Z score value. **B.** Immunofluorescence staining of β -catenin expression after CYD 6-17 treatment for 12 h in T24-P cells.

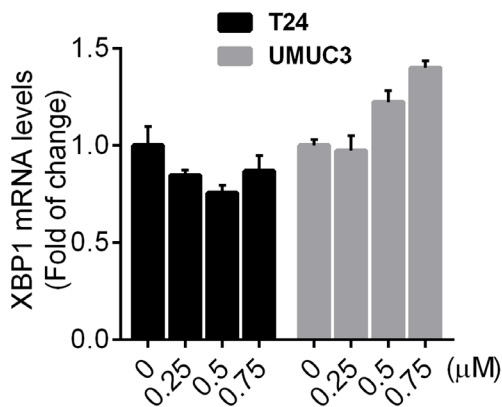
A



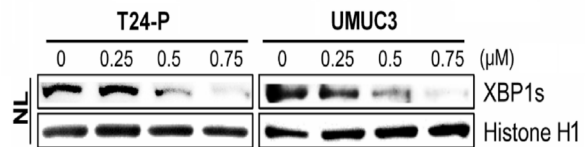
B



C



D



Supplementary Figure S2: A. Effect of CYD 6-17 on the mRNA expression from endogenous β-catenin or exogenous CA-β-catenin in UMUC3 cells. *, $p < 0.05$. **B.** XBP1 mRNA expression in TCC cells after CYD 6-17 treatment. **C.** Expression of β-catenin in nuclear and cytosol fractions in T24-P and UMUC3 cells transfected with CA-β-catenin or vector. **D.** Effect of CYD 6-17 on the expression of XBP1s in nuclear lysate (NL) in T24-P and UMUC3 12 h after treatment.