

Supplementary materials

Inhibition of cyclin D1 by novel biguanide derivative YB-004 increases the sensitivity of bladder cancer to Olaparib via causing G0 / G1 arrest

Di Xiao^{1, 3#}, Xuetong Chu^{1#}, Weifan Wang¹, Mei Peng¹, Qi Lv¹, Cangcang Xu^{1*}, Huaxin Duan^{1*}, Xiaoping Yang^{1, 2*}

¹Key Laboratory of Study and Discovery of Small Targeted Molecules of Hunan Province, Department of Oncology, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, The Research Center of Reproduction and Translational Medicine of Hunan Province, Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research of Ministry of Education, Department of Pharmacy, School of Medicine, Hunan Normal University, Changsha 410013, Hunan, China; ²FuRong Laboratory, Changsha 410078, Hunan, China; ³TCM and Ethnomedicine Innovation and Development International Laboratory, Innovative Material Medical Research Institute, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, China.

These authors contributed equally to this work.

*Corresponding author: Cangcang Xu (xucangcang@hunnu.edu.cn), Huaxin Duan (huaxinduan_123@sina.com), Xiaoping Yang (xiaoping.yang@hunnu.edu.cn)

27

28

Contents

29 Supplementary Figures.....pages 3-6

30 Supplementary Tables.....pages 7-8

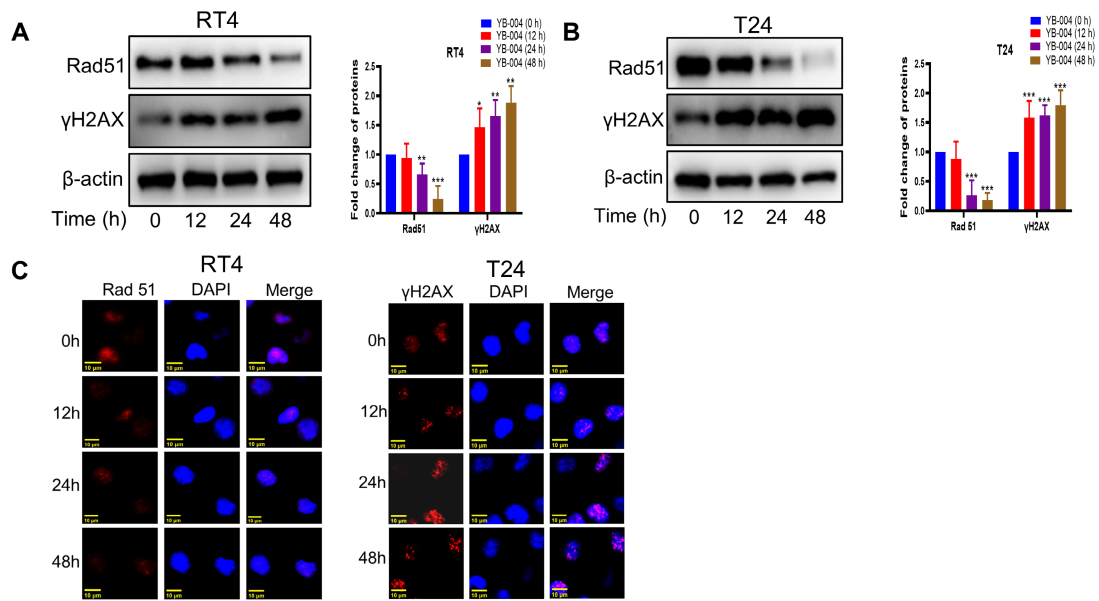
31

32

33

34

35 **Figure S1**



36

37 **Figure S1. YB-004 inhibited HR of HR-proficient BC cells exacerbating DNA damage. A-C.**

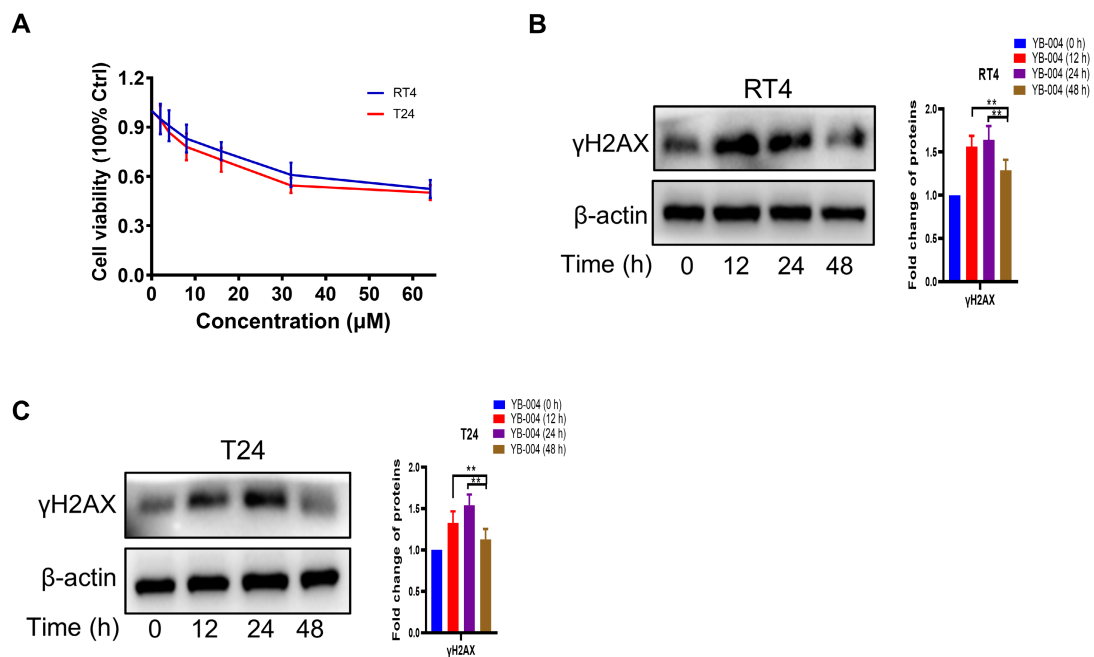
38 Cells were treated with **YB-004**, and the changes of the indicated proteins were analyzed by WB

39 and immunofluorescence (n=3, Error bars represent means ± SD from triplicate experiments, *P <

40 0.05; **P < 0.01; ***P < 0.001).

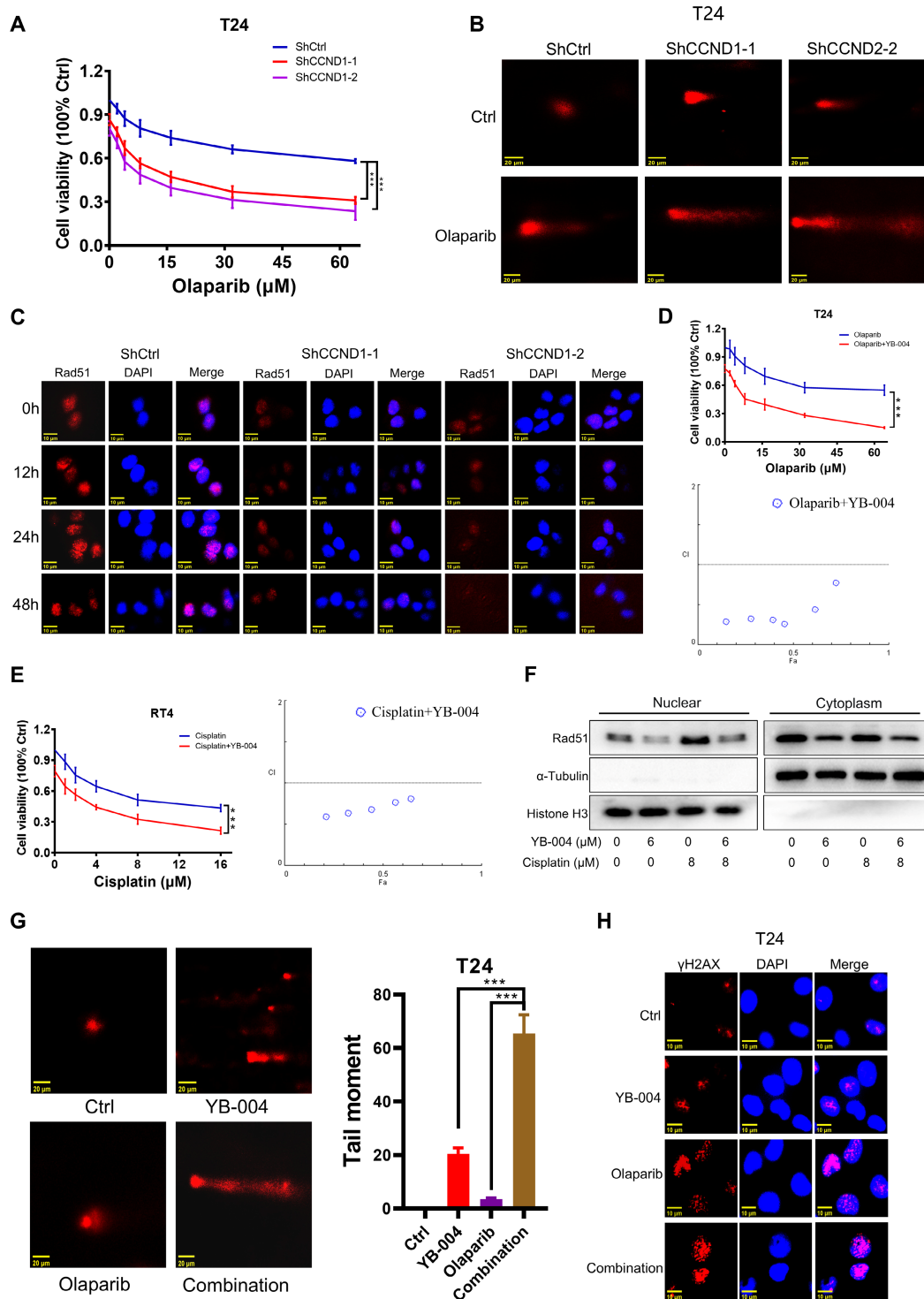
41

42 **Figure S2**



43

44 **Figure S2. The effects of Olaparib on bladder cancer cells. A.** The cell viability after Olaparib
45 treatment were evaluated by MTT assay. **B-C.** RT4 (B) and T24 (C) were treated with Olaparib,
46 and the changes of the indicated proteins were analyzed by WB (n=3, Error bars represent means
47 \pm SD from triplicate experiments, ****P** < 0.01).



49

50 **Figure S3. Combination of YB-004 and Olaparib synergistically inhibits the growth of**
 51 **HR-proficient BC cells in vitro.** **A.** The cell viability of T24 expressing shCtrl or shCCND1
 52 treated with Olaparib at different concentrations was evaluated by MTT. **B.** T24 expressing shCtrl
 53 or shCCND1 were treated with indicated concentrations of Olaparib for 24 h and the degree of
 54 DNA damage was measured by comet assay. **C.** BC cells expressing shCtrl or shCCND1 were

55 treated with Olaparib and the changes of the indicated proteins were analyzed by
56 immunofluorescence. **D.** The cell viability of T24 treated with **YB-004** and Olaparib alone or in
57 combination were evaluated by MTT. The combination index (CI) was calculated using
58 CompuSyn software. **E.** The cell viability of RT4 treated with **YB-004** and cisplatin alone or in
59 combination were evaluated by MTT. The combination index (CI) was calculated using
60 CompuSyn software. **F.** RT4 were treated with **YB-004** or cisplatin and separated into nuclear
61 and cytoplasmic fractions. These fractions were then detected by WB. **G.** T24 were treated with
62 **YB-004** and Olaparib alone or in combination for 24 h and the degree of DNA damage was
63 measured by comet assay. **H.** T24 were treated with **YB-004** and Olaparib alone or in combination
64 and the changes of the indicated proteins were analyzed by immunofluorescence (n=3, Error bars
65 represent means \pm SD from triplicate experiments, ***P < 0.001).
66

67 **Table S1. Primers for human genes in RT-PCR**

Gene	Forward Primer(F)	Reverse Primer(R)
CCND1	GCGGAGGAGAACAAACAG	GCGGTAGTAGGACAGGAA
CCND2	CCGACAACCTCCATCAAGCCTCAG	TGCCAGGTTCCACTTCAACTTCC
CCND3	AGCCTCAGGGAAGCCTCTCAG	CATCTGTAGGAGTGCTGGTCTGG
CCNE	TGTCCTGGATGTTGACTGCCTTG	TTCTCTATGTGCGCACCACTGATACC

68

69

70 **Table S2. shRNA sequences for the targeted genes**

Gene	Forward Primer(F)	Reverse Primer(R)
Sh-CCND1-1	CcggGAACAAACAGATCATCC	aattcaaaaaGAACAAACAGATC
	GCAACTCGAGTTGCGGATGAT	ATCCGCAACTCGAGTTGCGG
	CTGTTTGTTCTTTTTg	ATGATCTGTTTGTTTC
Sh-CCND1-2	CcggGCACGATTCATTGAACA	aattcaaaaaGCACGATTCATTG
	CTTCTCGAGAAGTGTTCAATG	AACACTTCTCGAGAAGTGTT
	AAATCGTGCTTTTTg	CAATGAAATCGTGC
Sh-NC	CCGGTTCTCCGAACGTGTCAC	AATTCAAAAATTCTCCGAAC
	GTTTCAAGAGAACGTGACACG	GTGTCACGTTCTCTTGAAAC
	TTCGGAGAATTTTTG	GTGACACGTTCCGGAGAA

71

72