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# Supplementary Materials for

## PP2Cδ inhibits p300-mediated p53 acetylation via ATM/BRCA1 pathway to impede DNA damage response in breast cancer

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## Supplemental materials

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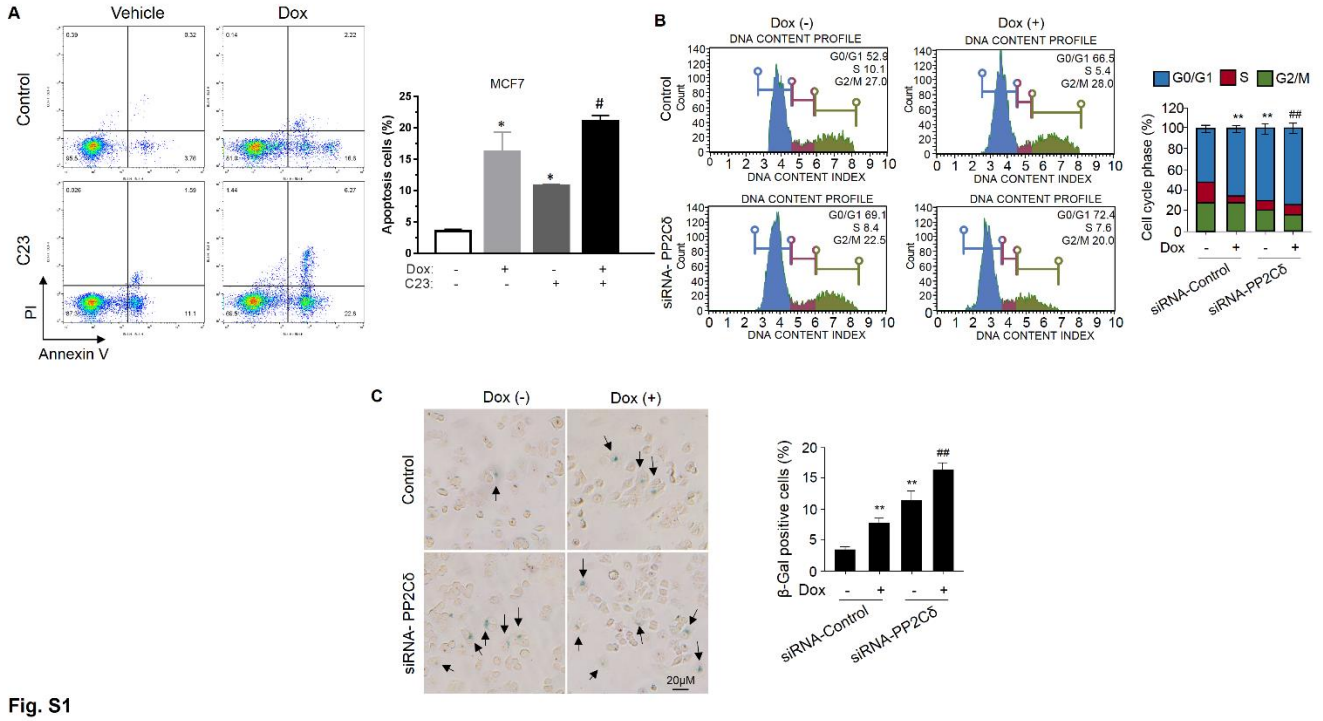


Fig. S1

**Fig. S1. Effect of PP2Cδ inhibition on apoptosis, cell cycle progression, and senescence in MCF-7 cells. A**. MCF-7 cells were pre-treated with or without 2.5 µM C23 for one hour, followed by incubation with Dox (1.0 µM) for 48 h. Cells were then collected and processed for apoptotic cell analysis using flow cytometry after annexin V-FITC/propidium iodide (PI) staining. An average from three replicates for each treatment (± SD) is shown. *p < 0.05 control; #p < 0.05 versus Dox alone. **B**. Cells were transfected with control or PP2Cδ siRNA for 24 h followed by incubation with Dox (1.0 µM) for 24 h, followed by analysis of cell cycle distribution using the Muse™ cell analyzer. Cell population in each cell cycle phase was numerically depicted. Notably, knockdown or inhibition of PP2Cδ significantly influenced the cells in the G0/G1 phase. An average from three replicates for each treatment (± SD) is shown. **p < 0.01 versus siRNA-control/Dox (-); ##p < 0.01 versus siRNA-control/Dox (+). **C**. The Senescence β-Galactosidase Staining Kit (Cell Signaling; #9860) was used in MCF7 cells to evaluate β-galactosidase activity at pH 6, a known characteristic of senescence. Images were taken under bright light for β-*Galactosidase staining at pH 6.0* on *MCF-7 cells* transfected with PP2Cδ siRNA or treated with Dox (1.0 µM) for 24 h as indicated. An enlarged cell size and pH-dependent β-galactosidase activity characterize senescent cells. An average from three replicates for each treatment (± SD) is shown. **p < 0.01 versus siRNA-control/Dox (-); ##p < 0.01 versus siRNA-control/Dox (+).

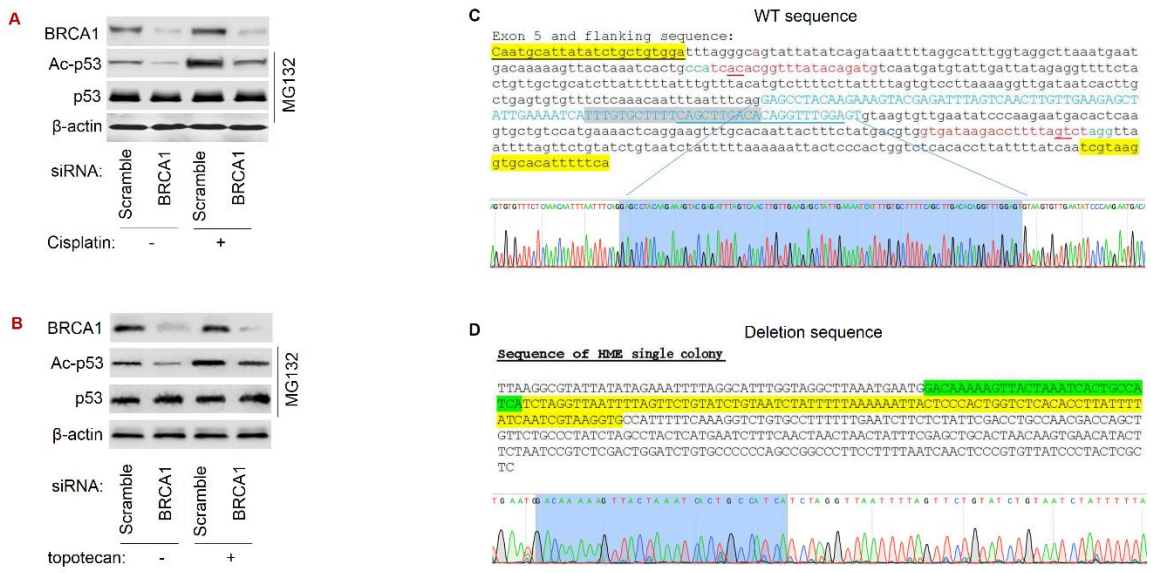


Fig. S2

**Fig. S2. p53 acetylation was decreased upon BRCA1 knockdown.** MCF-7 cells were transfected with scramble or BRCA1 siRNA, followed by cisplatin (50 μM for 24h) (A) or topotecan treatment (1 μM for 2h) (B). p53 protein level was normalized by MG132 (20 μM). p53 acetylation level on Lys373 and levels of BRCA1, p53 and β-actin were detected with specific antibodies. The data represent mean±SD from 3 separate experiments. **C and D. Sanger sequencing reveals the homozygous deletion of the BRCA1 exon5. C.** Wild type sequence: The shadow part is Exon 5 sequence, and the left and right sides of the shadow are introns of Exon 5. **D**. Deletion sequence: the shaded part corresponds to the sequence with green color on the top panel (the right end is the upstream gRNA targeted cleavage site) and the sequence following the shaded part corresponds to the sequence with yellow color on the top panel (the left end is the downstream gRNA targeted cleavage site).

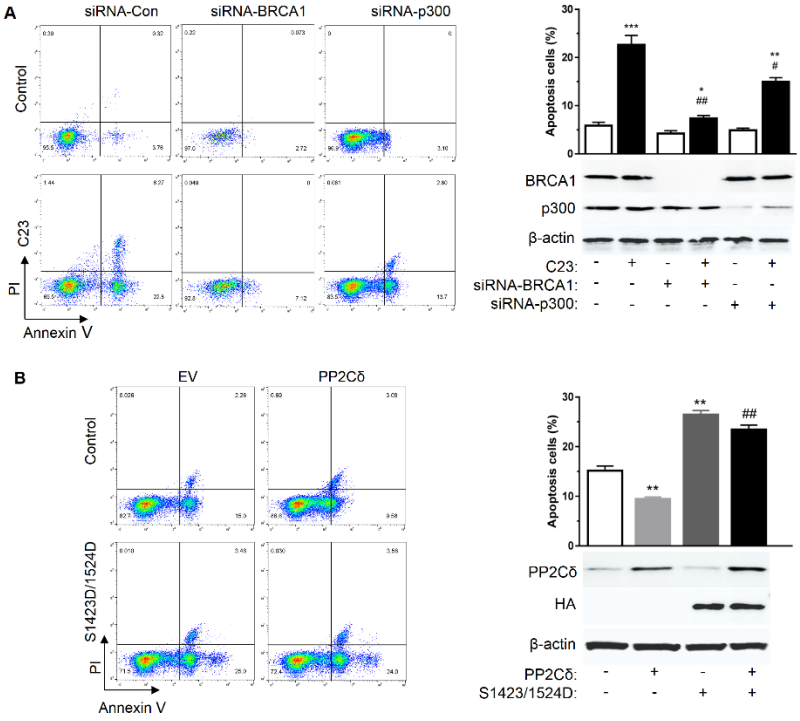


Fig. S3

**Fig. S3. Phosphorylation of BRCA1 on Ser$^{1423/1524}$ mediates apoptosis triggered by PP2Cδ inhibition. A**. MCF-7 cells were transfected with control siRNA , BRCA1 siRNA or P300 siRNA as indicated for 24 h followed by incubation with C23 (3.5 µM) for 48 h. Cells were then collected and processed for apoptotic cell analysis using flow cytometry after annexin V-FITC/propidium iodide (PI) staining. An average from three replicates for each treatment (± SD) is shown. *p < 0.05, **p < 0.01, ***p <0.001 versus siRNA-control/C23 (-); #p < 0.05, ##p < 0.01 versus siRNA-control/C23 (+). **B**. Human normal mammary epithelial cells (MCF-10A) transfected with empty vector (EV) or plasmid expressing wild type PP2Cδ (PP2Cδ-WT) or BRCA1 S1423/1524D as indicated, were exposed to doxorubicin (Dox) (0.1 µM) for 24 h. Cells were then collected and processed for apoptotic cell analysis using flow cytometry after annexin V-FITC/propidium iodide (PI) staining. An average from three replicates for each treatment (± SD) is shown. **p < 0.01 versus EV; ##p < 0.01 versus PP2Cδ(+).

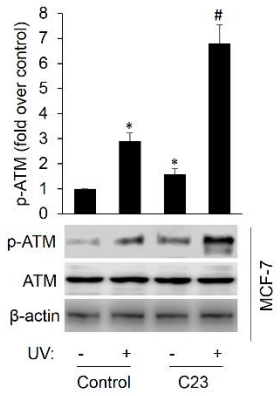


Fig. S4

**Fig. S4. C23 markedly increases basal and UV-induced ATM phosphorylation in MCF-7 cells.** MCF-7 cells were pre-treated with 2.5 µM C23 for one hour, followed by UV treatment (20 J/m2 for an 8-h recovery). Cells were lysed and the ATM phosphorylation and protein levels were detected by western blotting with specific antibodies. The data represent mean ± SD from 3 separate experiments. *P<0.05 versus Control/UV (-); #P<0.05 versus Control/UV (+).

**Table S1. Sequences for sgRNA oligos.**

| sgRNA oligos | Sequences |
|---|---|
| Guide #1: (reverse) specificity score =92, predicted efficiency score =70 | Forward: 5'-CACCGCATCTGTATAAACCGTGTGA-3'<br>Reverse: 5'-AAACTCACACGGTTTATACAGATGC-3' |
| Guide #2 (sense) specificity score =78, predicted efficiency score=44 | Forward: 5'-CACCGTGATAAGACCTTTTAGTCT-3'<br>Reverse: 5'-AAACAGACTAAAAGGTCTTATCAC-3' |