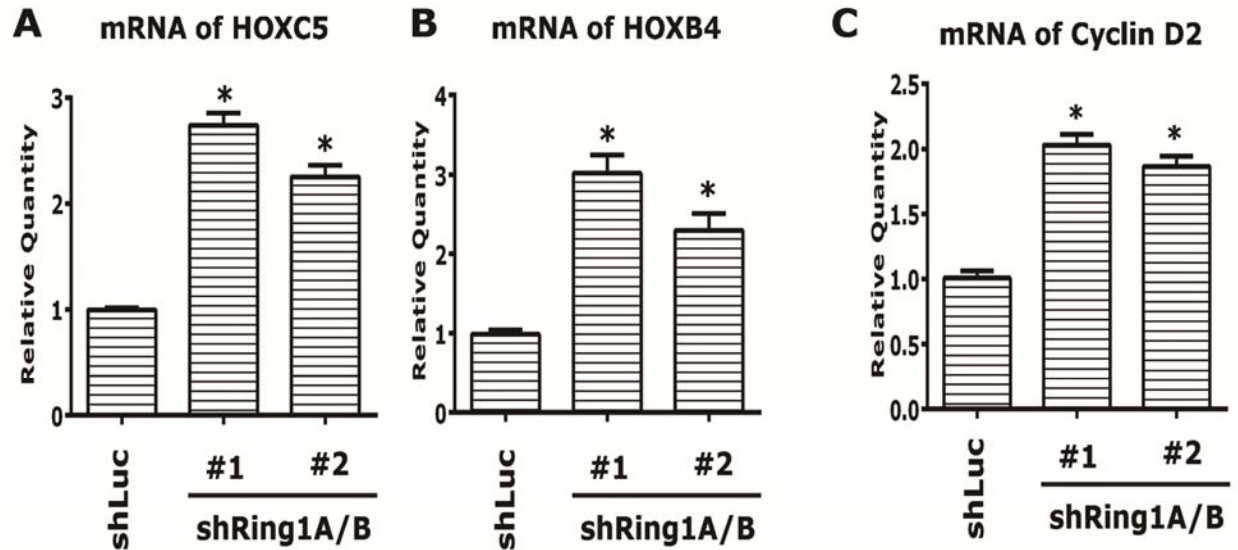
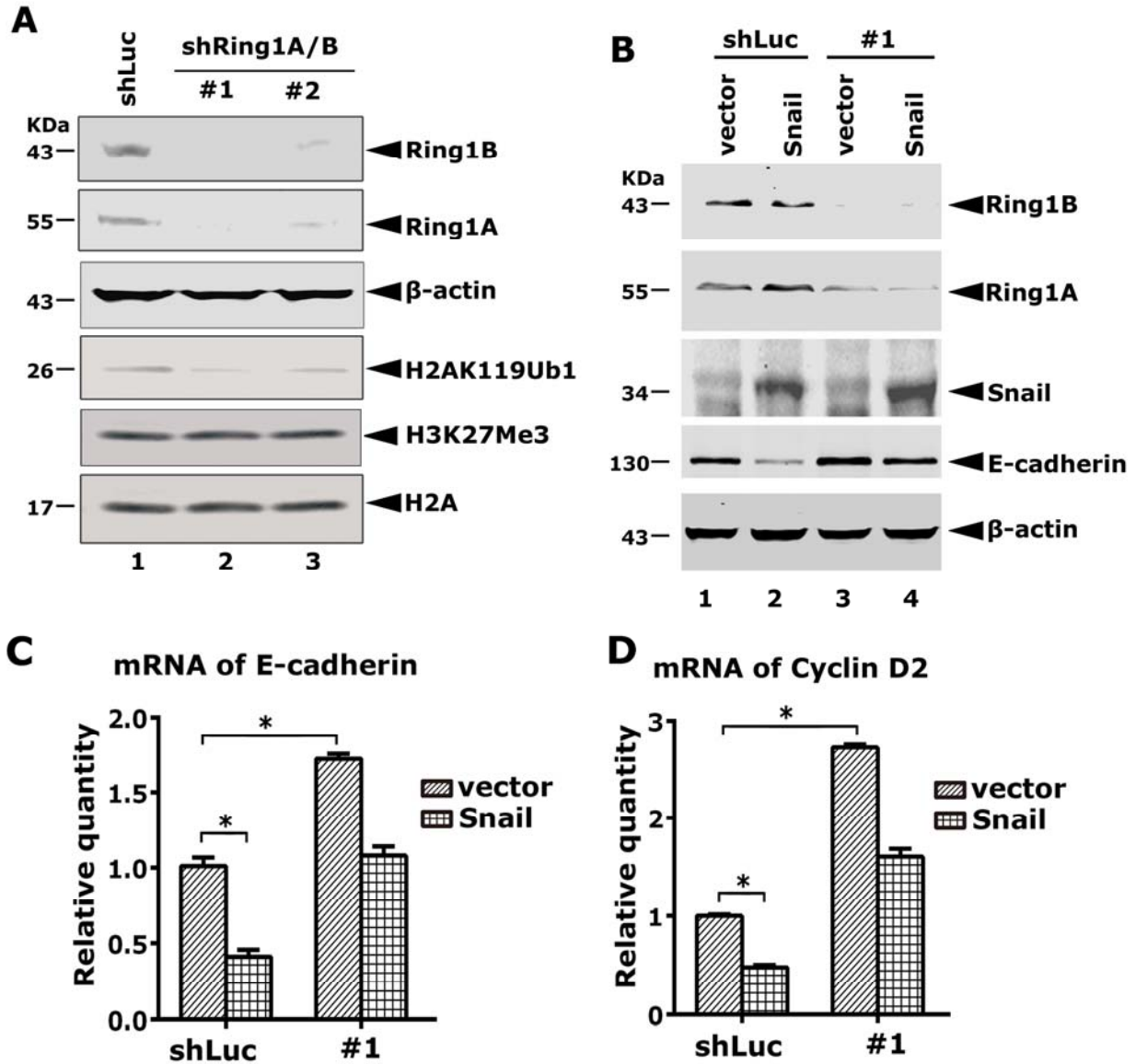


**S 1. Mutation of Ring domains of Ring1A/B proteins does not change their subcellular localization.** HA-Snail, Flag-Ring1A-WT or Ring1A-MT (H66A, C69A), or Ring1B-WT or Ring1B-MT (H69A, C72A) were transiently expressed in PanC1 cells and were detected by immunofluorescence staining with Flag or HA antibodies. Confocal imaging showed either Ring1A/B-WT (green) and Snail (red), or Ring1A/B-MT (green) and Snail (red), shared similar distribution in the nucleus (orange).



S2. qRT-PCR analysis of the known PRC1 target genes *HOXC5* and *HOXB4*(1) and Snail target gene *Cyclin D2*(2) in PanC1-shLuc and PanC1-shRing1A/B cells. GAPDH was used as loading reference. Data were presented as Mean  $\pm$ SD from 3 independent experiments. #1 and #2, different cell pools generated from Ring1A and Ring1B double knocking down by shRNAs; \* indicates statistical significance,  $P < 0.05$ . **Primers used for real-time PCR are as follow:**

Human *HOXC5*, forward 5-TAAGCAGAGCCCCAATATCCC-3, and reverse 5-CCAATCCGCCGTAGCAGTAC-3; Human *HOXB4*, forward 5-TCACGTGAGCACGGTAAAC-3, and reverse 5-CAGGTAGCGGTTGTA GTGAAA-3; Human *Cyclin D2*, forward 5-CTGTGTGCCACCGACTTTAAGTT-3, and reverse 5-GATGGCTGCTCCCACACTTC-3; Human *Snail*, forward 5-CCTCCCTGTCAGATGAGGAC-3, and reverse 5-CCAGGCTGAGGTATTCCTTG-3; Human *E-cadherin*, forward 5-TGCCAGAAAATGAAAAAGG-3, and reverse 5-GTGTATGTGGCAATGCGTTC-3.



**S3. Simultaneous depletion of Ring1A and Ring1B results in global decrease of H2AK119 monoubiquitination, and Snail loss of repression on E-cadherin in AsPC1 cells. (A)** Ring1A and Ring1B were knocked down simultaneously in AsPC1 cells by specific shRNAs via viral infection. Western blot showed Ring1A and Ring1B were depleted in AsPC1 cells. Similar to that observed in PanC1 cells, depletion of Ring1A/B resulted in global decrease of H2AK119Ub1, but no obvious change on H3K27Me3. **(B)** Snail was stably expressed in AsPC1-shLuc or AsPC1-shRing1A/B cells. E-cadherin was reduced when Snail was expressed in

AsPC1-shLuc cells, but was not changed in AsPC1-shRing1A/B cells. Whole cell lysate (100µg) was loaded and β-actin was used as loading control. **(C, D)** qRT-PCR analysis of Snail target genes E-cadherin and Cyclin D2 in AsPC1-shLuc and AsPC1-shRing1A/B cells. GAPDH was used as loading reference. Data were presented as Mean ±SD from 3 independent experiments. #1 and #2, different cell pools generated from Ring1A and Ring1B double knocking down by shRNAs; \* indicates statistical significance, P<0.05.

## **Reference**

1. del Mar Lorente M, Marcos-Gutiérrez C, Pérez C, Schoorlemmer J, Ramírez A, Magin T, et al. Loss-and gain-of-function mutations show a polycomb group function for Ring1A in mice. *Development*. 2000;127:5093-100.
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