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Supplemental Information

Dual role of deubiquitinating enzyme USP19 regulates mitotic progression and tumorigenesis by stabilizing survivin

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Supplemental figures

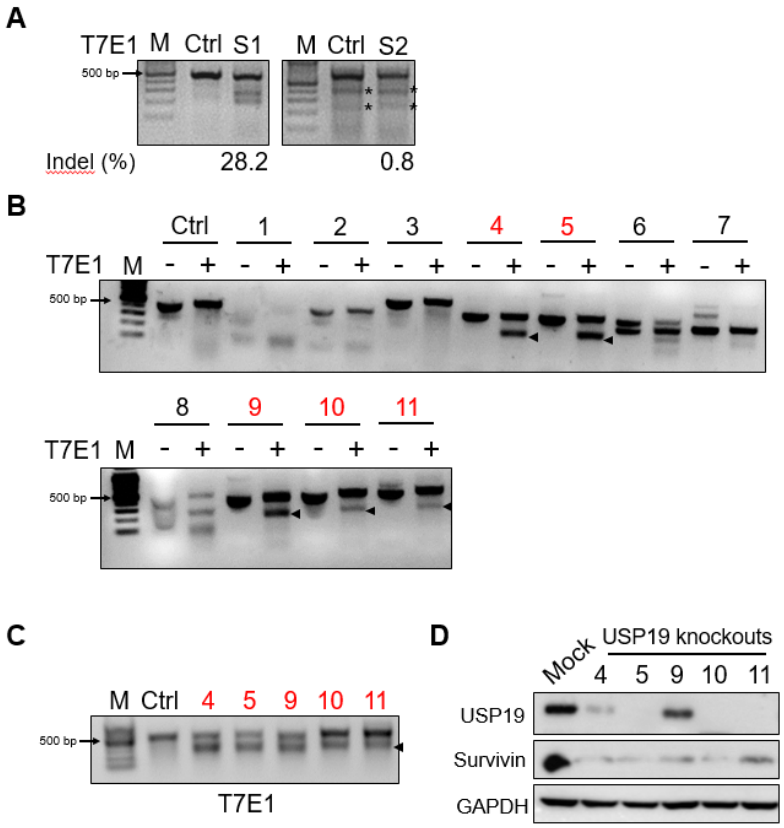

Figure S1. Generation of single cell-derived USP19 knockout clones in HCT116 cell line. (A) Knockout efficiency of the designed sgRNAs was validated in HCT116 cell line by T7E1 assay. Untransfected cells served as negative control (Ctrl), and the size marker (M) is shown. Asterisk denotes non-specific bands. (B) Screening of stable USP19 knockout clones in HCT116 cells by T7E1 assay. T7E1 positive clones showing cleavages are represented in red text. Arrowheads show the cleavage site. (C) Reconfirmation of positive USP19 knockout clones by T7E1 assay. Untransfected cells served as negative control (Ctrl), and the size marker (M) is shown. Arrowheads show the cleavage site. (D) Western blot analysis shows the knockout efficiency of USP19 in HCT116 cells and the effect of USP19 knockout in survivin protein levels.

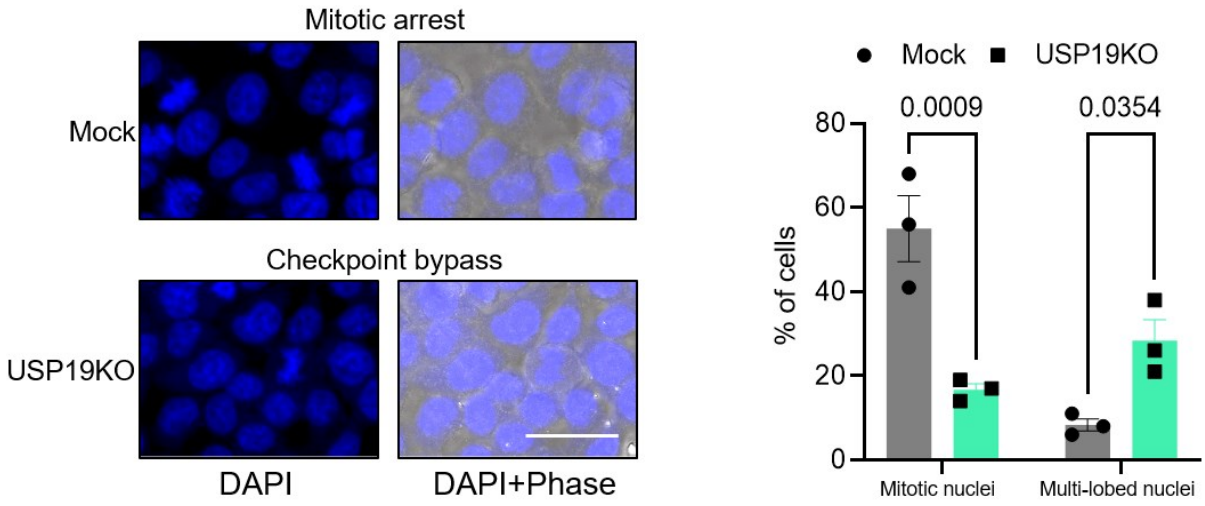

Figure S2. The loss of USP19 leads to mitotic checkpoint bypass in Taxol-treated cells. Mock or USP19KO HCT116 cells were treated with Taxol for 24 h. Nuclei were stained with DAPI (Left). The HCT116 cells with nuclear abnormalities were quantified after nuclear staining (Right). 100 cells per group were examined from three independent experiments. Scale = 25 μm.

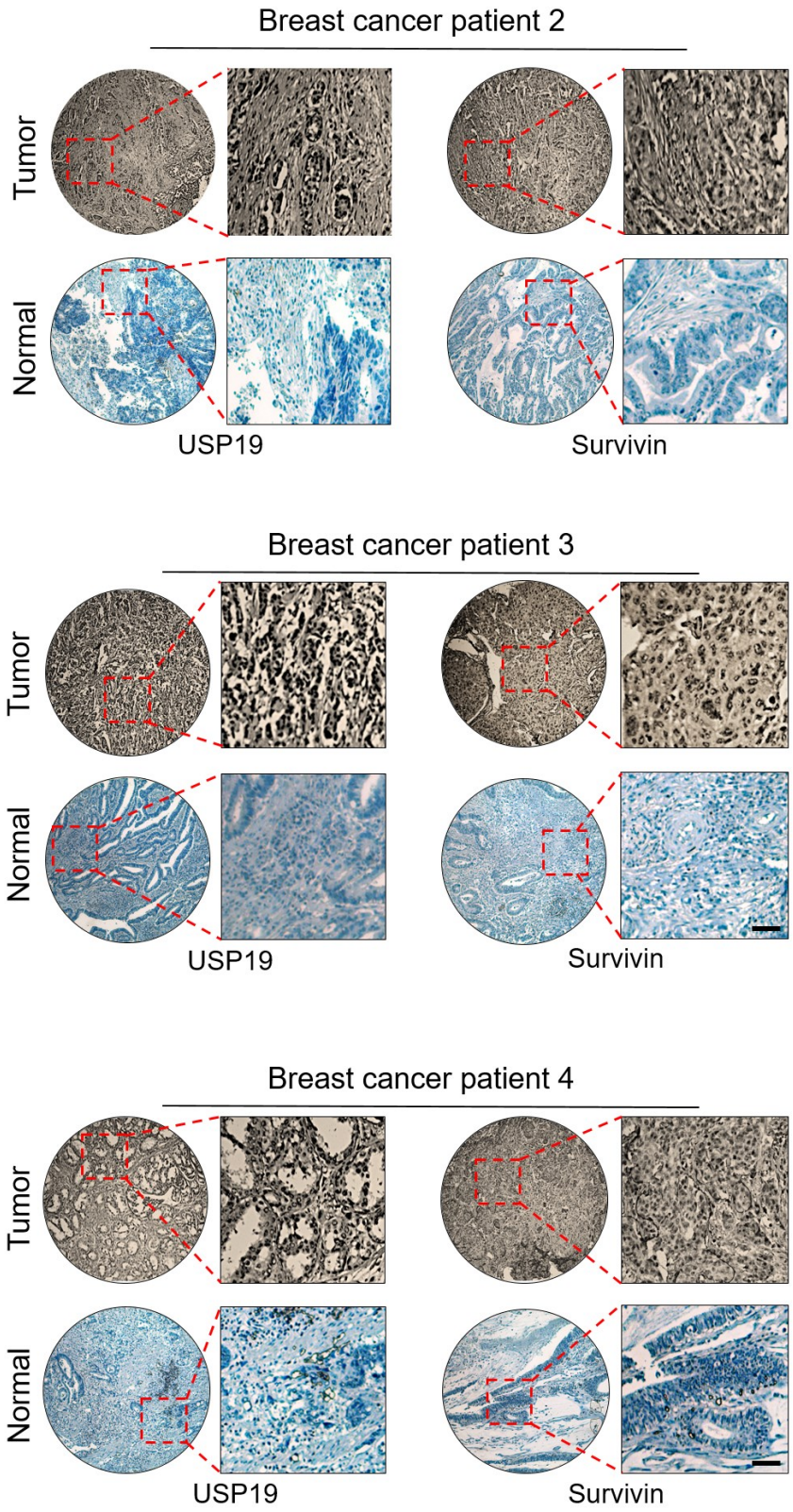

Figure S3. Immunohistochemical staining of USP19 and survivin in human breast cancer tissues. Scale bar = 25 μm.

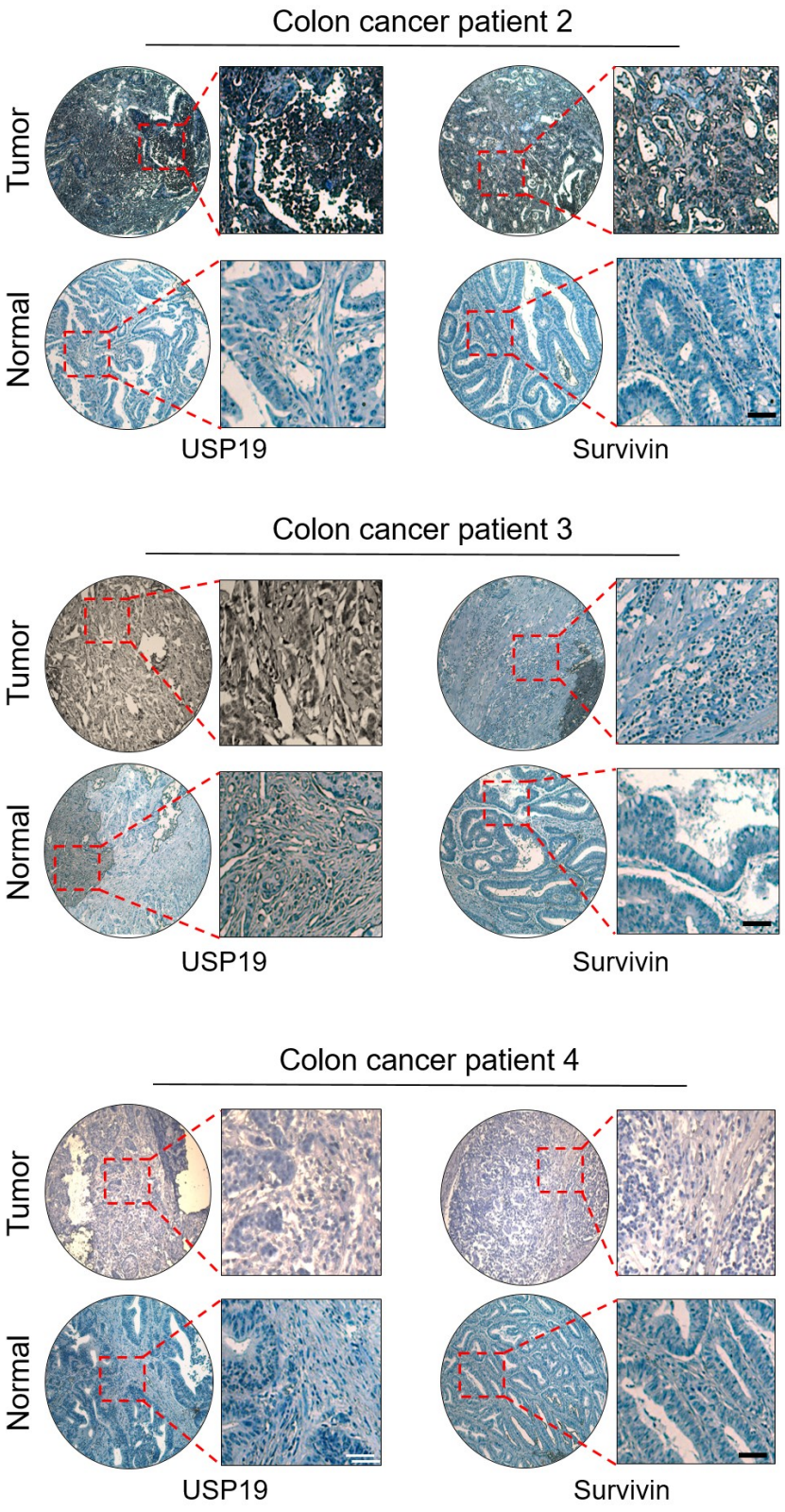

Figure S4. Immunohistochemical staining of USP19 and survivin in human colon cancer tissues. Scale bar = 25 μm.

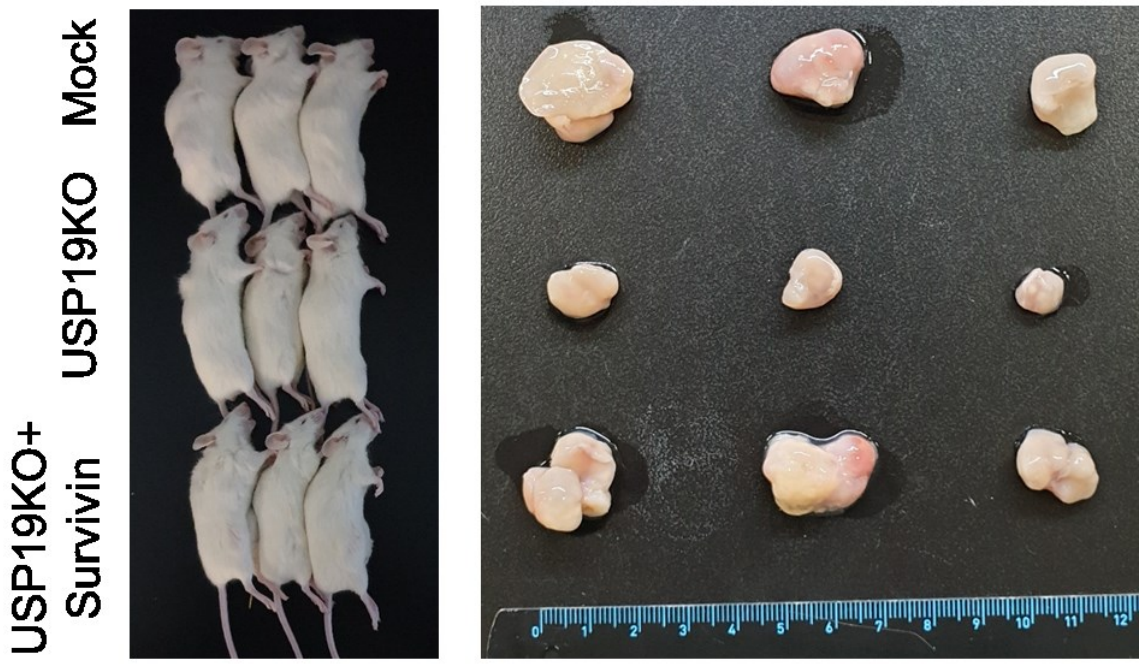

Figure S5. USP19KO inhibits tumor progression *in vivo*.

Xenografts were generated by subcutaneously injecting the mentioned cell groups into the right flank of NSG mice (n = 3). Tumor volumes were recorded and stored for IHC experiments. The right panel shows the tumors excised from the mice after the experiment.

Supplemental tables

Table S1. Expression values of USP19 and survivin mRNA from the DepMap portal.

Table S2. mRNA expression values of USP19 and survivin in different cancer tissues from the GEPIA 2 database.

Table S3. mRNA expression values of USP19 in different cancer tissues.

Table S4. The sample size of Tumor and Normal tissues for USP19 gene obtained from GENT2 database.

Table S5. Oligonucleotides used for sgRNA plasmid construction.

Supplemental videos

File name: Supplemental Video 1

Description: Mitosis in Mock HCT116 cells. GFP-H2B was transfected in Mock HCT116 cells and then treated with thymidine. Green fluorescence was to visualize chromosome movement. This video shows normal mitosis. These images were taken every 3 min and they are played back at 5 frames per second. Scale bar = 10 μm.

File name: Supplemental Video 2

Description: Mitosis in USP19KO HCT116 cells. GFP-H2B was transfected in USP19KO HCT116 cells and then treated with thymidine. Green fluorescence was to visualize chromosome movement. This video displays the presence of misaligned chromosomes and mitotic delay during mitosis. These images were taken every 3 min and they are played back at 5 frames per second. Scale bar = 10 μm.

File name: Supplemental Video 3

Description: Mitosis in Mock HCT116 cells. GFP-H2B was transfected in Mock HCT116 cells and then treated with thymidine. This video displays the normal mitosis. These images were taken every 3 min and they are played back at 5 frames per second. Scale bar = 10 μm.

File name: Supplemental Video 4 and 5

Description: Mitosis in USP19KO HCT116 cells. GFP-H2B was transfected in USP19KO HCT116 cells and then treated with thymidine. This video displays the chromosome misalignments, chromosome bridges, and lagging chromosomes. These images were taken every 3 min and they are played back at 5 frames per second. Scale bar = 10 μm.

File name: Supplemental Video 6

Description: Mitosis in survivin reconstituted in USP19KO HCT116 cells. GFP-H2B was transfected in USP19KO+survivin HCT116 cells and then treated with thymidine. This video displays the normal mitosis. These images were taken every 3 min and they are played back at 5 frames per second. Scale bar = 10 μm.

File name: Supplemental Video Movie 7

Description: Mitosis and cytokinesis in Mock HCT116 cells. GFP-H2B was transfected in Mock HCT116 cells and then treated with thymidine. This video displays normal cytokinesis. These images were taken every 3 min and they are played back at 5 frames per second. Scale bar = 10 μm.

File name: Supplemental Video 8

Description: Mitosis and cytokinesis in USP19KO HCT116 cells. GFP-H2B was transfected in USP19KO HCT116 cells and then treated with thymidine. This video displays cytokinesis failure. These images were taken every 3 min and they are played back at 5 frames per second. Scale bar = 10 μm.