

Type of file: figure

Label: Fig S1

Filename: Fig. S1 final.psd

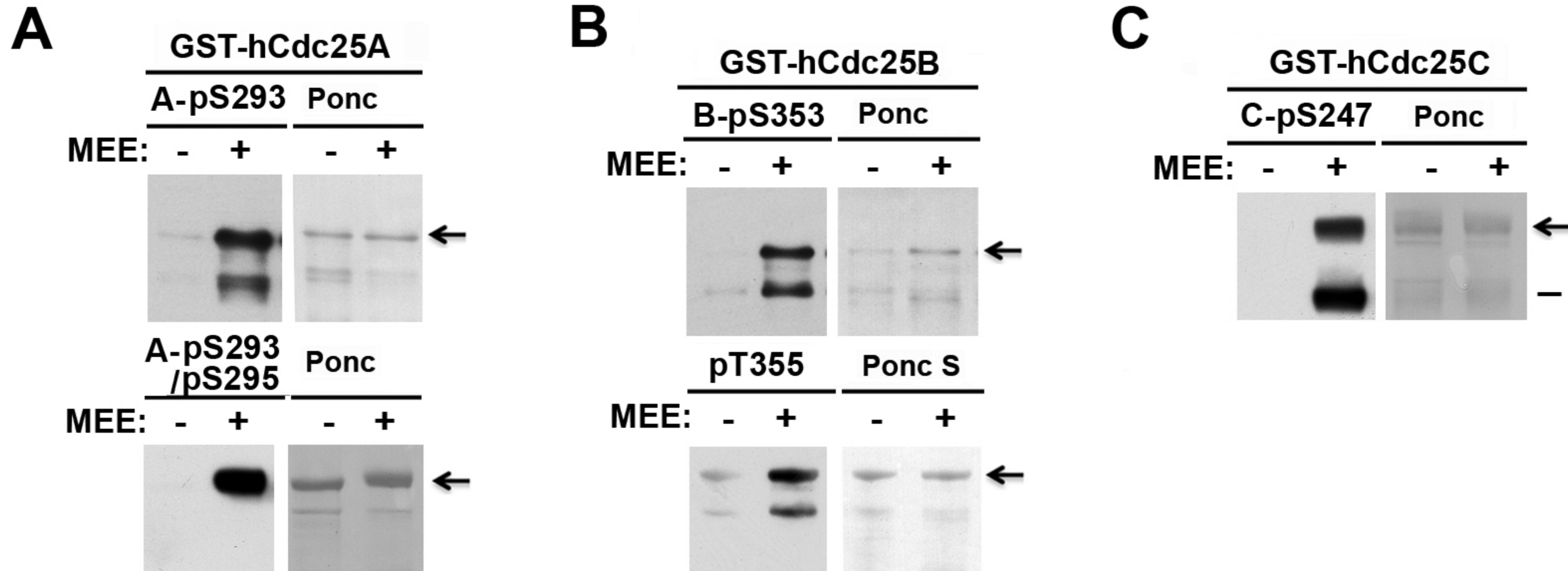


Figure S1. Xenopus egg extracts phosphorylates the predicted RSK sites in all three isoforms of hCdc25 with high efficiencies. The immobilized GST-tagged hCdc25A (A), hCdc25B (B) or hCdc25C (C) was mock-treated or phosphorylated with 1:5-diluted MEE at room temperature for 30 min. Proteins eluted from washed beads were immunoblotted with each of the indicated phospho-specific antibodies after Ponceau S (Ponc) staining.

Type of file: figure

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Type of file: figure

Label: Fig S3

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Type of file: figure

Label: Fig S4

Filename: Fig. S4 final.psd

A**RSK-1-siRNA-1**

Sense: 5'-GGCAACGCUGAAAGUACGUdTdT-3'
 Antisense: 5'-ACGUACUUUCAGCGUUGCCdTdT-3'

RSK-1-siRNA-2:

Sense: 5'-GUGGGCACCUGUAUGCUAUdTdT-3'
 Antisense: 5'-AUAGCAUACAGGUGCCCACdTdT-3'

RSK-2-siRNA-1:

Sense: 5'-GGCCACACUGAAAGUUCGAdTdT-3'
 Antisense: 5'-UCGAACUUUCAGUGUGGCCdTdT-3'

RSK-2-siRNA-2:

Sense: 5'-GGAGGAGAUUAACCCACAAdTdT-3'
 Antisense: 5'-UUGUGGGUUAUCUCCUCCdTdT-3'

Firefly luciferase GL3 siRNA (negative control)

Sense: 5'-CUUACGCUGAGUACUUCGAdTdT-3'
 Antisense: 5'-UCGAAGUACUCAGCGUAAGdTdT-3'

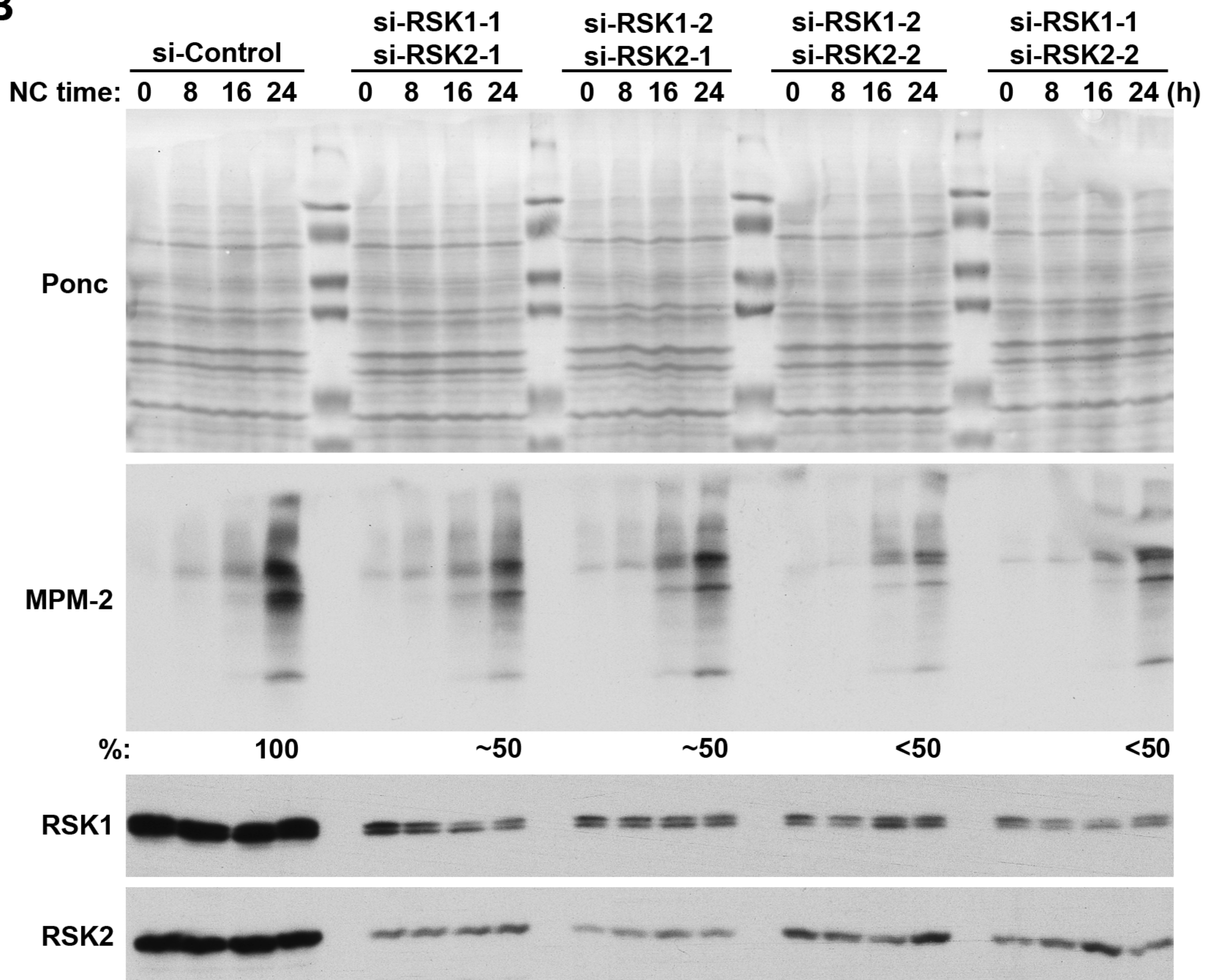
B

Figure S4. Knockdown of RSK1/2 expression delays G2/M transition in PC-3mm2 cells. (A) Sequences of the siRNA used in this experiment. (B) PC-3mm2 cells were transfected twice with indicated siRNAs within 36 hrs, and cells were subcultured in between the two transfections. These cells were then treated with TDR for 36-48 hrs, followed by release of the TDR block and addition of the nohodazole (NC) block. At indicated hours after NC addition, lysates of these cells were prepared and immunoblotted with MPM-2 after Ponceau S (Ponc) staining. Expressions of RSK1 and RSK2 were determined by separate immunoblotting.

Type of file: figure

Label: Fig S5

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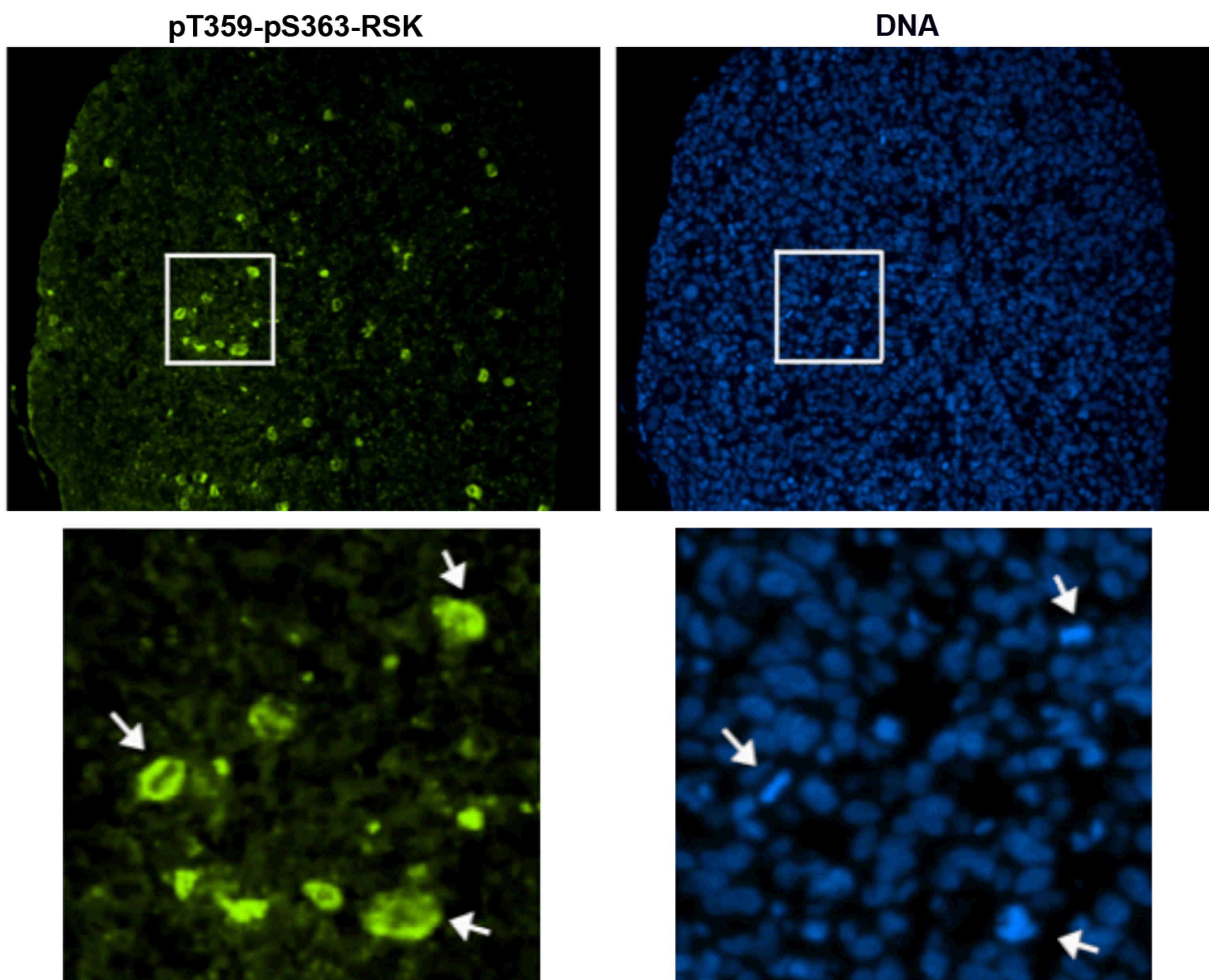


Figure S5. The phospho-specific antibodies for phosphorylated T359-S363 in RSK preferentially stain mitotic cells in PC-3mm2 tumors formed in nude mice. PC-3mm2 cells were implanted subcutaneously to form tumors in nude mice, and a paraffin section of a xenograft tumor were stained with the phospho-specific antibodies for phosphorylated T359-S363 in RSK and counterstained with the DNA dye DAPI. Images in the lower panel are enlarged images of squares in the upper panel. Arrows indicate mitotic cells.

The NIHMS has received the file 'Supplemental Materials final.docx' as supplementary data. The file will not appear in this PDF Receipt, but it will be linked to the web version of your manuscript.