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SUPPLEMENTARY ONLINE DATA

Human RASSF7 regulates the microtubule cytoskeleton and is required for spindle formation, Aurora B activation and chromosomal congression during mitosis

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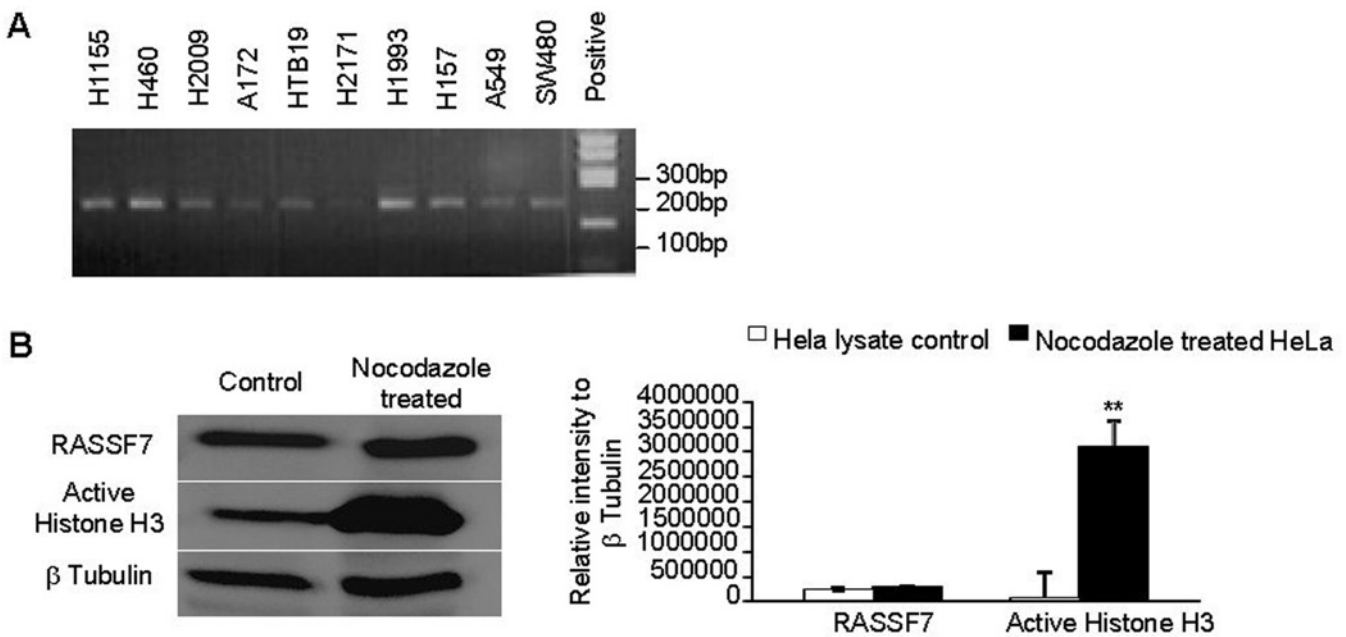

Figure S1 Expression of *RASSF7*

(**A**) Methylation of the *RASSF7* promoter. Analysis of the methylation status of the 5′ CpG island associated with the *RASSF7* gene was carried out using COBRA (combined bisulfite restriction analysis). The full-length PCR product was 206 bp and complete digestion with BstUI would generate products less than 86 bp. Efficient enzyme activity is indicated by complete digestion of plasmid DNA (positive control). No methylation was observed in any of the cancer cell lines analysed (20 lung, 12 breast, eight colorectal, eight kidney, five glioma and four neuroblastoma). Data from ten cell lines are presented. Sequencing of samples identified the correct PCR product and confirmed the unmethylated state. (**B**) Expression of RASSF7 protein in mitosis. HeLa cell lysates were enriched with M-phase cells through nocodazole treatment (75 ng/ml) for 18 h followed by mitotic shake off. There was no significant change in RASSF7 protein expression levels. Expression of the positive control, active histone H3 expression, increased as expected. $^{**}P < 0.01$ compared with corresponding controls.

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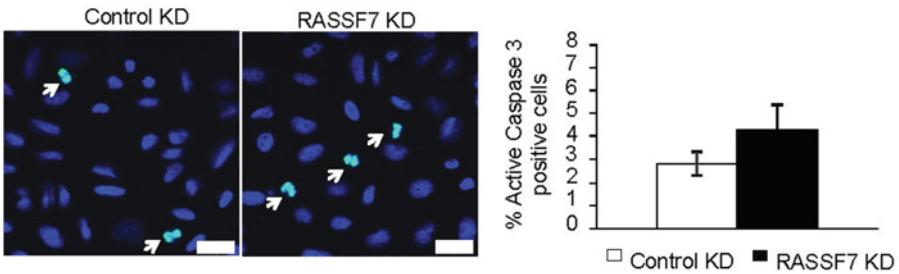

Figure S2 *RASSF7* knockdown did not cause a significant increase in apoptosis

RASSF7 depletion in HeLa cells did not significantly increase the number of active caspase 3-positive cells (green, highlighted by arrows) compared with controls. More than 500 cells were counted for each sample from three independent experiments. Blue shows nuclear staining. Scale bar, 20 μm.

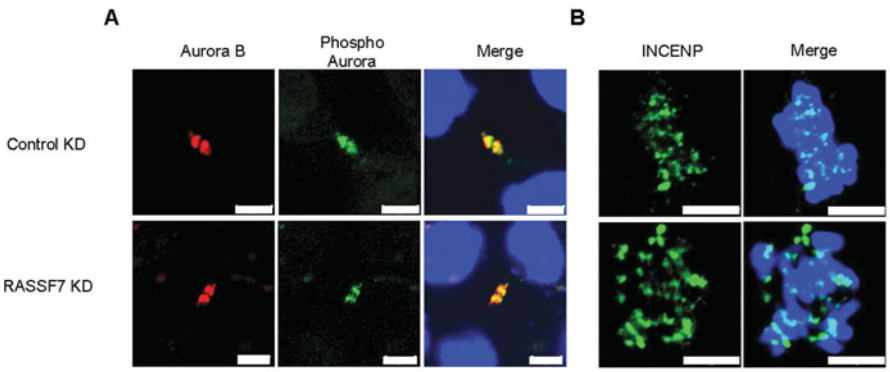

Figure S3 Aurora B activity during mitosis

(**A**) Aurora B phosphorylation appears normal during cytokinesis in *RASSF7*-knockdown HeLa cells. (**B**) INCENP (green), which is required for Aurora B activation, maintains its correct localization during metaphase in *RASSF7*-knockdown HeLa cells. Blue shows nuclear staining. Scale bar, 5 μm.

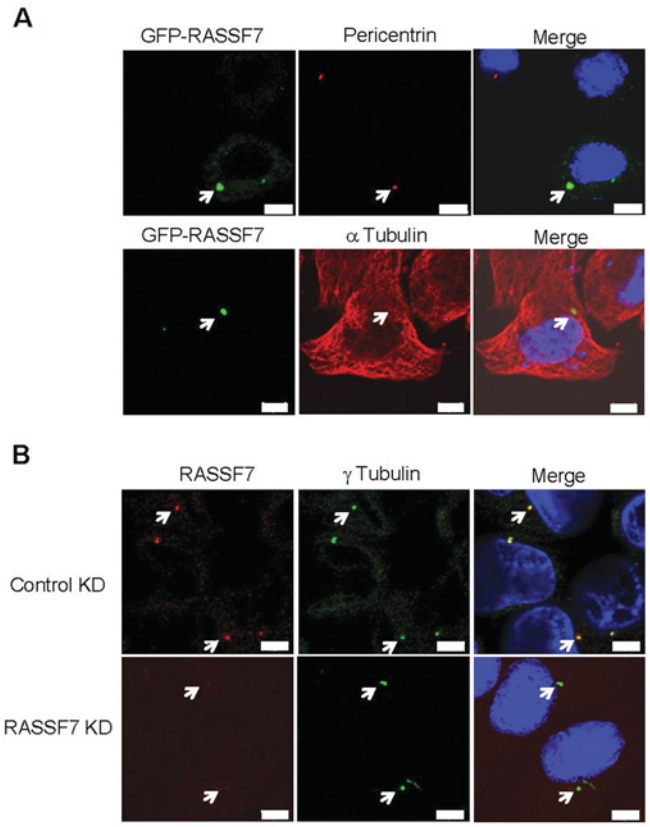

Figure S4 Localization of RASSF7

(**A**) A GFP–RASSF7 fusion protein localizes to the centrosomes (arrows), marked with pericentrin, when expressed at low levels in HeLa cells. When expressed at high levels, GFP–RASSF7 formed large aggregates (results not shown). We did not see any stabilization of microtubules by RASSF7 (high or low levels), reminiscent of the striking phenotype seen after expressing RASSF1A. (**B**) The endogenous centrosomal RASSF7 staining is lost in *RASSF7*-knockdown HeLa cells (arrows). Blue shows nuclear staining. Scale bar, 5 μm.

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