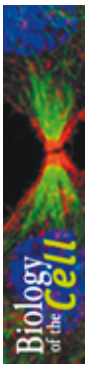
Biol. Cell (2011) **103**, 55–68 (Printed in Great Britain) doi:10.1042/BC20100111

online data



Supplementary online data

CDC25B associates with a centrin 2-containing complex and is involved in maintaining centrosome integrity

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See the pages that follow for Supplementary Figures S1–S6.

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Figure S1 | Ctn depletion causes loss of CDC25B from the centrosome in U2OS cells

U2OS cells were treated with control or Ctn2 siRNA duplexes for 48 h before immunofluorescence detection of Ctn (green) and CDC25B (CDC25B-S230P, red) as in Figure 2(**A**). Examples of Ctn2-depleted cells demonstrating a loss of centrosomal CDC25B in some cells (arrows), in comparison with cells treated with control siRNA duplexes. (**B**) Percentage of U2OS cells in which CDC25B-S230P and Ctn2 (Ctn2 siRNA only) were not detectable or substantially diminished from the centrosomes. Bars represent means for at least 200 cells counted from three independent experiments ± S.D.

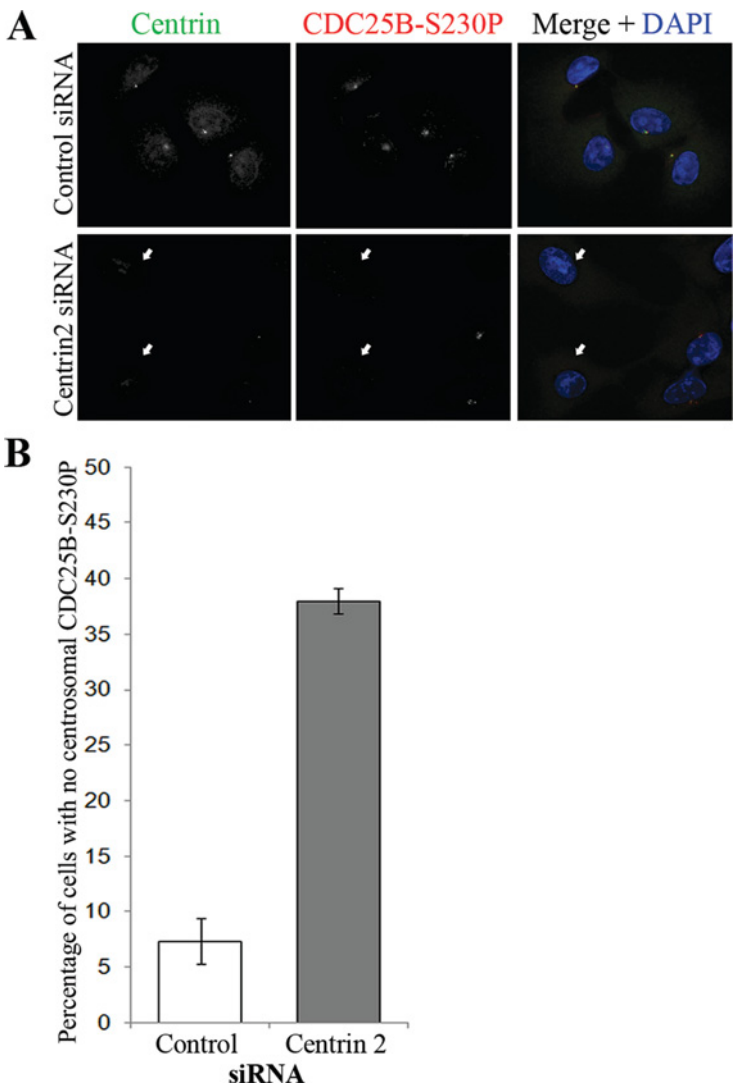


Figure S2 | Ctn depletion does not affect the centrosomal localization of Chk1

HeLa cells were treated with control or Ctn2 siRNA duplexes as in Figure 2 of the main paper before immunofluorescence detection of Ctn and Chk1. Percentages of HeLa cells in which Chk1 was not detectable or substantially diminished at the centrosomes are shown. Bars represent means for at least 200 cells counted from six independent experiments, performed using either Smartpool (Dharmacon) or custom (11) Ctn2 siRNA duplexes (± S.D.).

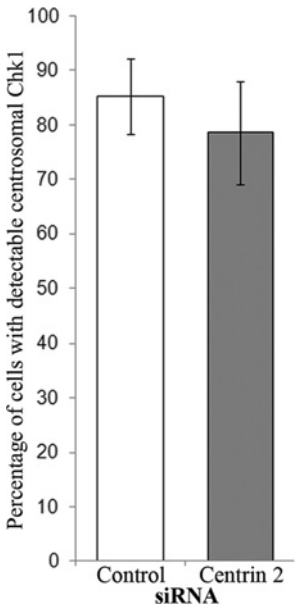


Figure S3 | Raw FRET–FLIM data showing reduced GFP-Ctn1 fluorescence lifetime when co-expressed with WT but not R506L mutant mOrange-CDC25B

(**A**) Lifetime distributions of GFP-Ctn with or without co-expression of mOrange constructs, comparing the fluorescence lifetime distributions of control HeLa-Ctn1 cells (GFP-Ctn1 fluorescence) with cells expressing the various mOrange control and fusion proteins. Each histogram represents the total number of cells displaying each fluorescence lifetime value, with the fluorescence mean lifetime shift due to FRET being clearly evident with co-expression of the mOrange-CDC25B. (**B**) Fluorescence decay curve for GFP-Ctn corresponding to the centrosome region, without (orange line) or with co-expression of mOrange (yellow triangles), mOrange-CDC25B (green line) or mOrange-CDC25BR506L (blue line) fusion proteins.

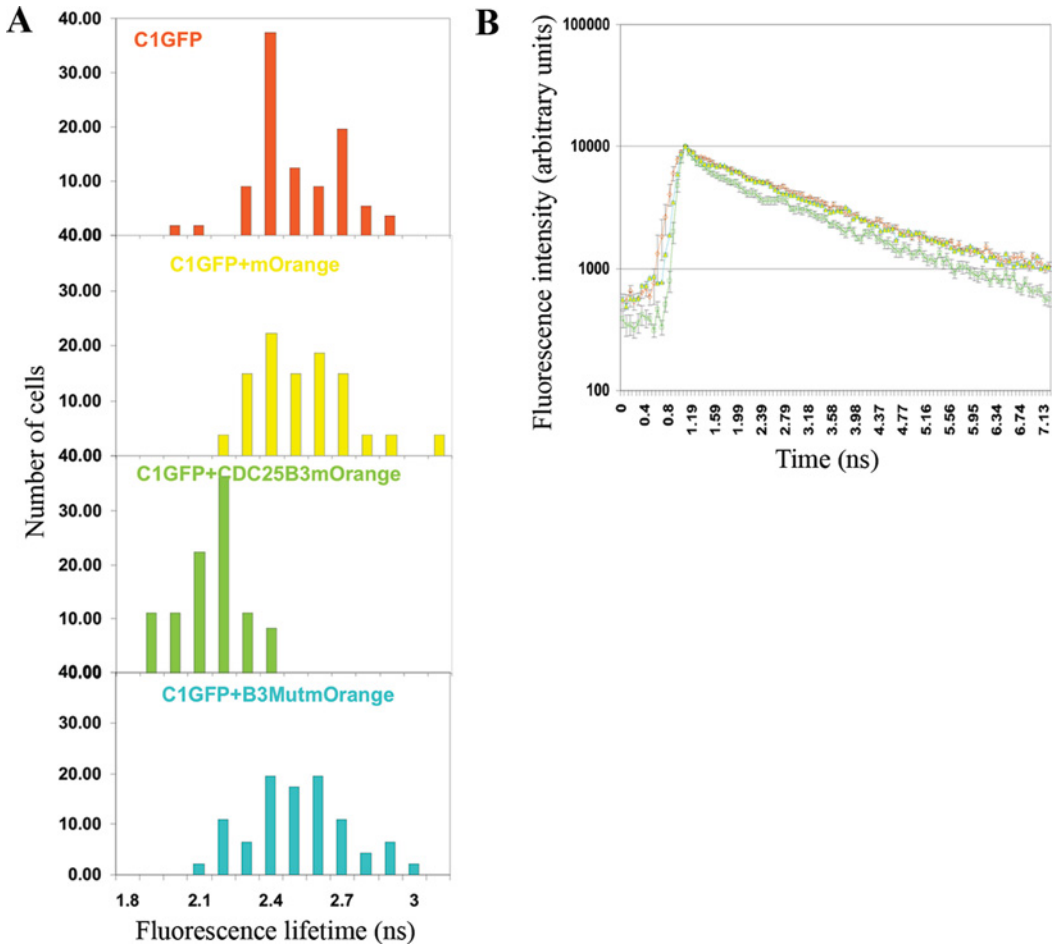


Figure S4 | CDC25B and Ctn1 co-immunoprecipitate

Endogenous CDC25B was immunoprecipitated (IP) from soluble protein extracts from asynchronous, G_1, S and G_2/M cell populations, using Protein G Sepharose in the presence (+) or absence (−) of IgG. Western blots (WB) were performed using anti-CDC25B and -GFP antibodies.

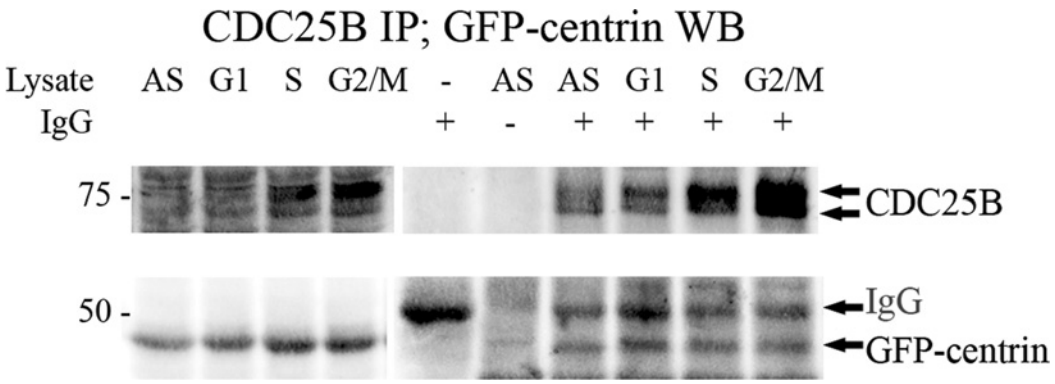


Figure S5 | CDC25B does not interact directly with Ctn1, Ctn2 or Ctn3

GST or GST-full-length WT CDC25B (WT FL 25B) fusion proteins produced in bacteria were bound to GST beads and incubated with recombinant *in vitro* translated [^{35}S]methionine-incorporated Ctn1, Ctn2, Ctn3 or luciferase (Lucif.) proteins prepared using rabbit reticulocyte extracts. Luciferase was used as positive control and a no DNA (– DNA) reaction was included as a negative control. Proteins attached to the GST beads were denatured and separated by SDS/PAGE. Gels were Coomassie Brilliant Blue-stained (Coomassie, lower gel) and then dried on to blotting paper and exposed to a phosphorimaging screen for a maximum of 2 weeks (^{35}S signal, upper gel).

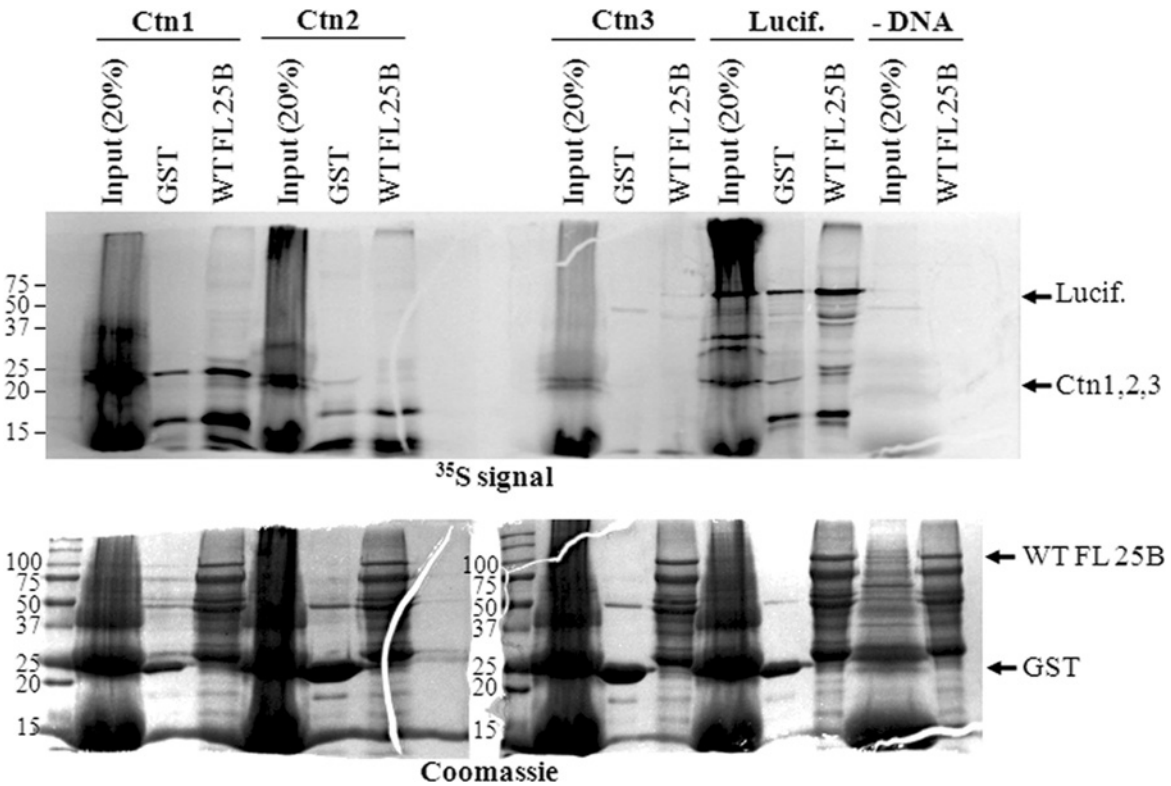
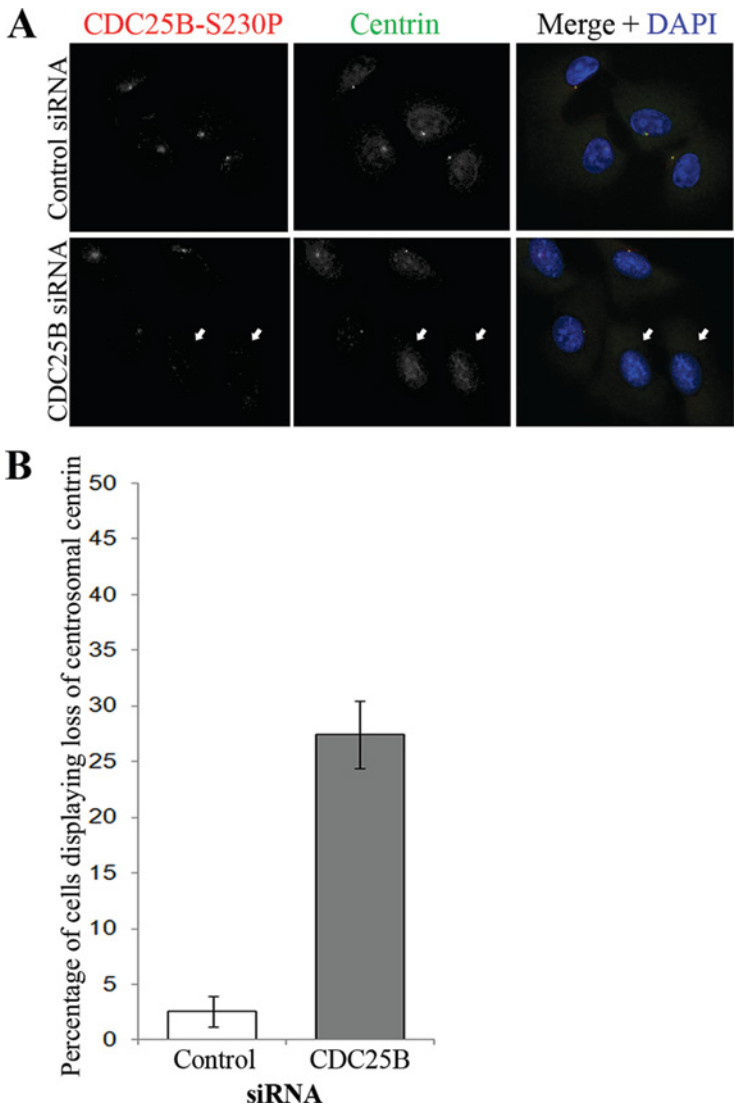


Figure S6 | CDC25B depletion causes loss of Ctn from the centrosome in U2OS cells

U2OS cells were treated with scrambled control or CDC25B siRNA duplexes as in Figure 5 of the main text. (**A**) Examples of siRNA-treated cells from which CDC25B was successfully depleted, which also show a loss of centrosomal Ctn2 (arrows). (**B**) Percentage of HeLa cells displaying loss of both CDC25B-S230P (CDC25B siRNA only) and Ctn2 from the centrosomes. Bars represent means for at least 200 cells counted from three independent experiments ± S.D.



Received 9 September 2010/19 November 2010; accepted 23 November 2010
Published as Immediate Publication 23 November 2010, doi:10.1042/BC20100111