Supplementary Figure Legends

Supplementary Figure 1 CHC constructs that contain residues 1109-1128 were recognised by X22 antibody.

CHC (X22/Alexa546) immunoreactivity is plotted against GFP fluorescence for constructs with (A) or without (B) CHC residues 1109-1128. Results are mean pixel density measurements from 8-bit images (0-255) of interphase cells from one experiment. Black line shows a line fit for all data points on each plot. For constructs with the X22 epitope (CHC residues 1109-1128; 1-1675, 1-1639, 1-1516, 1-1597, C1573S and 331-1639), a correlation could be seen between GFP expression (amount of epitope expressed) and X22 signal (A). Whereas for those constructs lacking the epitope (GFP, 1-479, Stunted, StuntedΔtripod and Stunted(Ii)), there was no correlation (B). This result argues against the possibility that the X22 immunoreactivity found in those cells expressing CHC constructs with the epitope represents endogenous CHC that is re-expressed following RNAi. Moreover, we found differential rescue of CME and mitosis by various CHC constructs, which did not correlate with presence or absence of X22 immunoreactivity.

Supplementary Figure 2 Differential rescue of kinetochore fibre stability by CHC constructs in cells depleted of endogenous CHC.

Representative confocal images of each construct expressed in HEK293 cells where endogenous CHC had been depleted. For each construct, a metaphase-like cell is shown (left) together with a zoomed view of a kinetochore pair from the cell equator (right). Anti-α-tubulin/Alexa546 (green), anti-CENP-B/Alexa647 (red) and

DNA/H33342 (blue). Scale bar, 5 and 2.5 μm for main and zoomed panels, respectively. In this qualitative assay of kinetetochore fibre stability, non-stable kinetochore fibres (those that do not have attachment to both the pole and the kinetochore) are depolymerised by treatment with cold media. This results in orphan kinetochore pairs at the cell equator in cells with kinetochore fibres which are destabilized. In Control cells, all kinetochores had stable kinetochore fibre attachments remaining, whereas in GFP cells, orphan kinetochores without stable attachment were found at the cell equator. We found evidence for rescue of kinetochore fibre stability in cells expressing 1-1675, 1-1639, CHC-ALK, Stunted and Stunted (Ii). There was no evidence of rescue in cells expressing GFP, 1-479, 1-1516, 1-1597, C1573S, 331-1639 and Stunted Δtripod; that is, orphan kinetochore pairs were frequently observed. It must be remembered however, that this is a qualitative assay and it can therefore only complement the more sensitive, quantitative assays (mitotic index and frequency of misaligned chromosomes) used in the main paper.

Supplementary Figure 3 Expression levels of CHC constructs did not correlate with rescue of mitotic defects.

Histogram to compare levels of GFP fluorescence for each of the CHC constructs used in this study. Results are mean ± sem, taken from 26-47 interphase cells per construct and are shown normalized to Control. We found equivalent levels of GFP fluorescence in interphase and M-phase cells (data not shown).