Fig. S1. Immunofluorescence staining of CtBP1 and CtBP2 expression in MCF-7 cells 4 d post-transfection with either control (top row) or CtBP siRNA (bottom row). Bar, 10 μm.

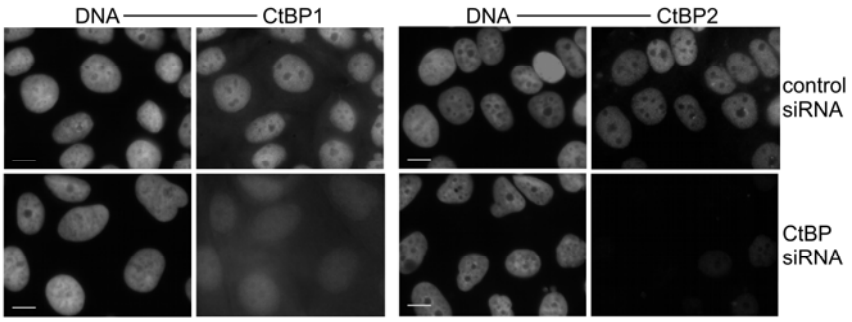

Fig. S2. Immunofluorescence analysis of phospho-histone H3 (red) in MCF-7 cells 4 days post transfection with control siRNA. DNA is stained with DAPI. A G_2 cell is indicated with an arrow. The strongly staining cell is in mitosis. Bar, 10 μm

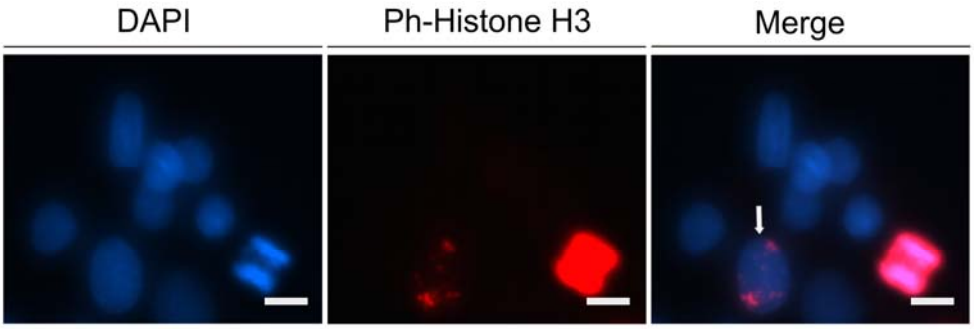

Fig. S3. Immunofluorescence analysis of CENP-A in MCF-7 cells 4 d post-transfection with control (top row) or CtBP (middle row) siRNA. As a positive control for micronuclei formed from DNA damage, MCF-7 cells were radiated with 5 Gy and fixed 48 h later (bottom row). White arrows indicate micronuclei (DAPI, blue) that show positive staining for the kinetochore protein CENP-A (red) suggesting that they are formed from whole chromosomes. Bar, 10 μm.

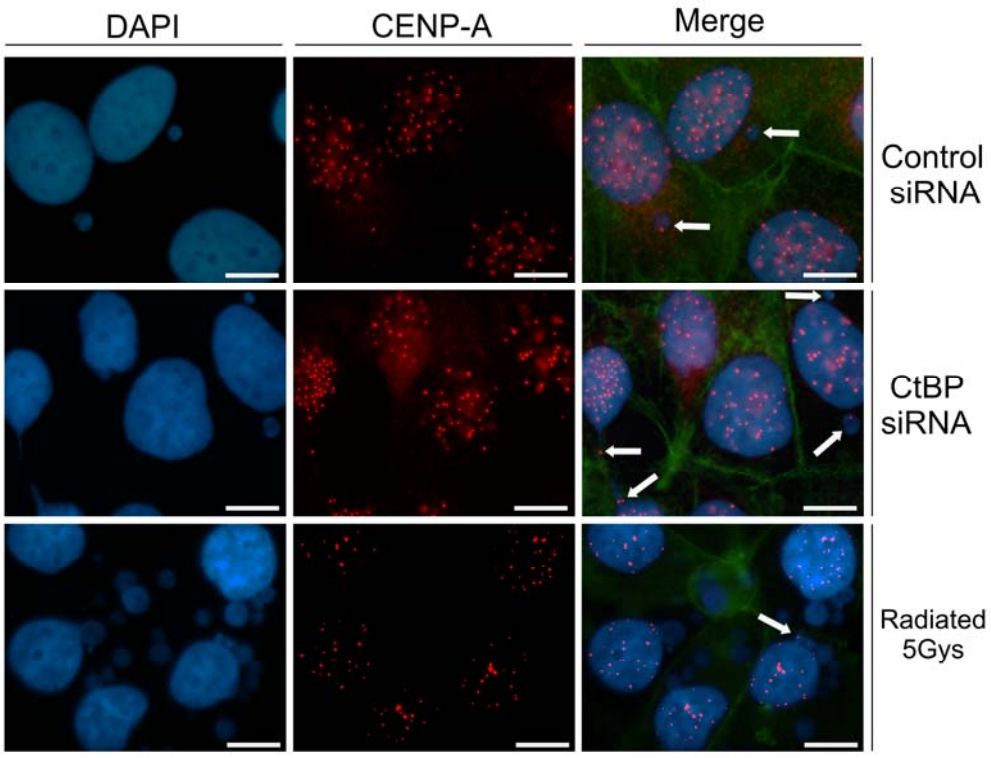

Fig. S4. Structure of the Golgi apparatus in CtBP-depleted cells. 4 d post-transfection, MCF-7 cells were immuno-stained with antibody to golgin-97 (A-21270; Molecular Probes). Images of representative cells in interphase and metaphase are shown. Bar, 10 μm. White arrows indicates abnormal nuclei, demonstrating that Golgi structure is normal in cells in which CtBP-depletion has resulted in an abnormal mitotic phenotype.

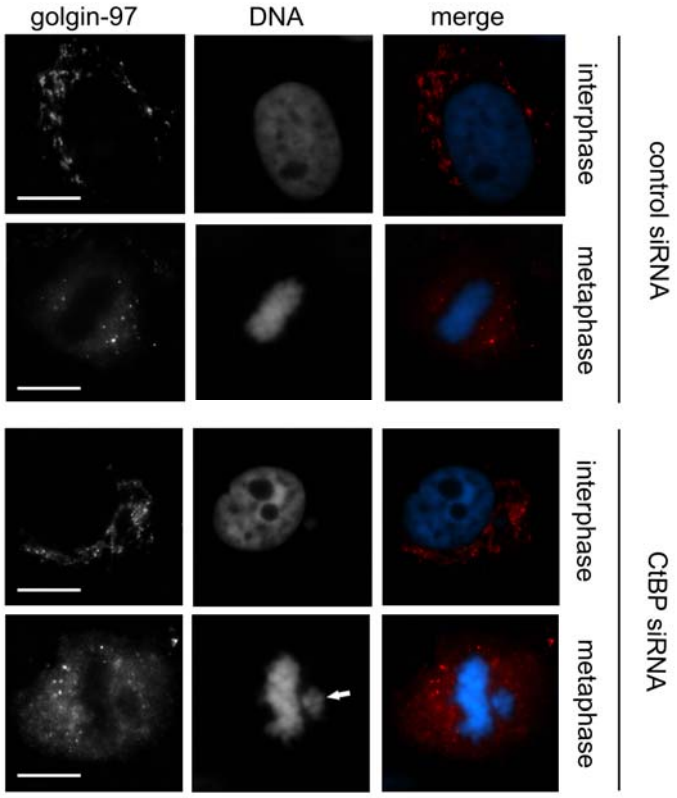

Fig. S5. Immunofluorescence images of MCF-7 cells 3 days post-transfection with control (top row) or CtBP (bottom row) siRNA, stained with antibody ab15246 (Abcam; manufacturers instructions were used for staining with this antibody) to α-tubulin, CtBP2 antisera, and DAPI. There is a small amount of wavelength overlap from FITC (α-tubulin) in the TRITC (CtBP2) channel. Merged images show localisation of α-tubulin (green) in relation to DNA (blue). Bar, 10 μm. Coverslips were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min, washed with PBS and incubated with ice cold methanol for 6 min. Cells were permeabilised and blocked with 1% BSA/ 0.03% Triton X-100/ PBS for 30 min.

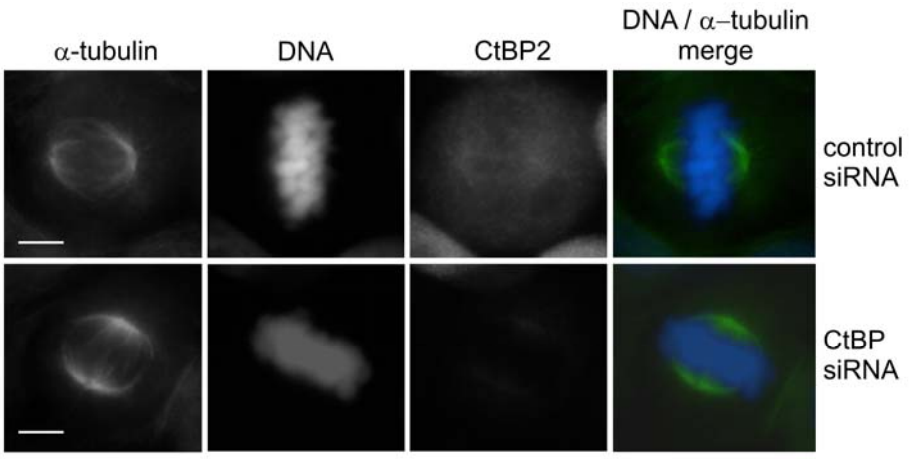

Fig. S6. Monoclonal antibody E12 is specific for CtBP1, and does not recognise CtBP2. MCF-7 cells were transfected with a. pcDNA3.1mh vector, b. CtBP1mh or c. CtBP2mh, and then probed with antibodies E12 and E16.

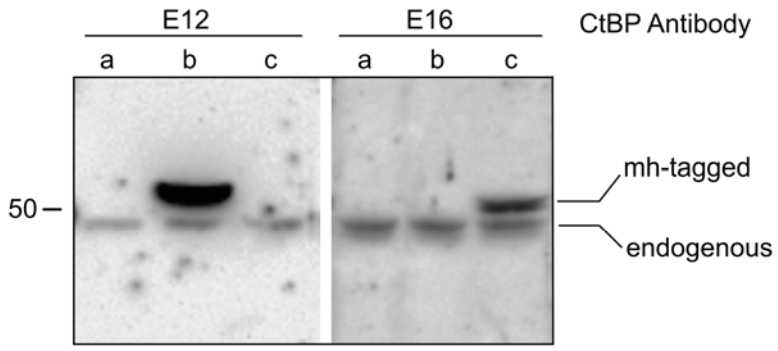

Fig. S7. hTERT-immortalised normal human MRC5 fibroblasts were transfected with either control siRNA (black bars) or CtBP siRNA (open bars) and analysed by western blotting for CtBP and p53 protein abundance (A, 48 h post transfection) or DAPI staining for frequency of cells containing micronuclei (B, 96 h post transfection).

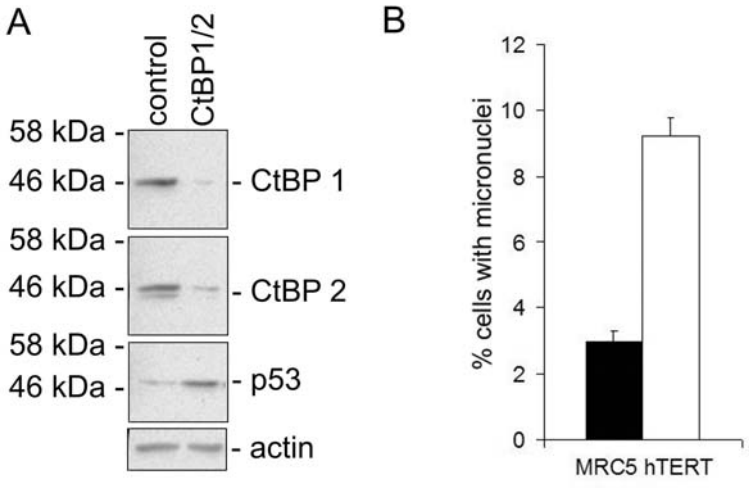

Fig. S8. Immunofluorescence staining of BubR1 (red) in prometaphase MCF-7 cells 4 d post transfection with either control or CtBP siRNA. Bar 10 μM. Antibody 8G1 to BubR1 was from AbCam. The overall pattern and intensity of staining was similar between control and CtBP-depleted cells, consistent with the SAC being activated in CtBP depleted cells throughout prometaphase.

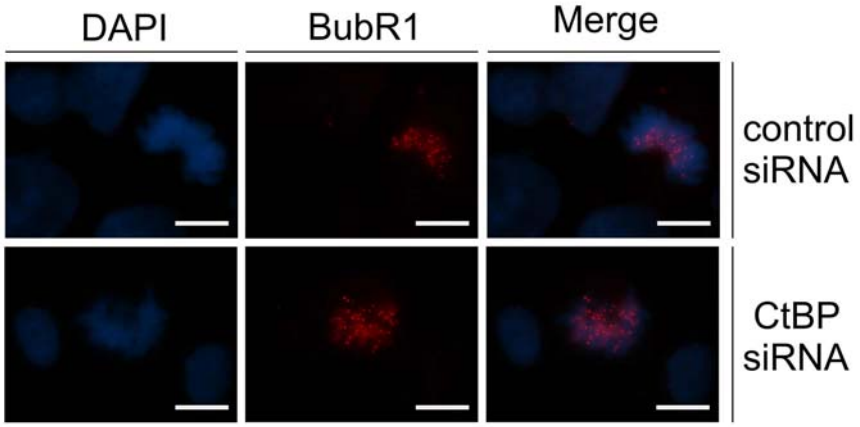

Fig. S9. MCF-7 cells were transfected with the indicated combinations of control, CtBP and p53 (Ambion siRNA ID s605) siRNA. This p53 siRNA targets a different sequence in p53 than that used in Fig. 6; results obtained with the two different p53 siRNAs were very similar. (A) Cells were lysed for western blotting 3 days after transfection. (B) Cells were analysed by live cell imaging for a 24 hour period from 2.5 to 3.5 days post transfection. Results from two representative fields for each combination of siRNAs are shown. Combining CtBP and p53 siRNAs results in reduced cell numbers and increased cell death compared to individiual siRNAs