Inventory of Supplemental Material

6 figures

2 Supplemental tables

Supplemental Figure 1 – related to Figure 1

Multiple sequence alignment of the N-terminal regions of human Cyclin A1, its paralog Cyclin A2 and their metazoan orthologs. Proteins were aligned with Clustal Omega and visualized in Jalview.

Supplemental Figure 2 – related to Figure 2

ABBA motif peptides bind to human CDC20 expressed in insect cells. Hs-CDC20 was expressed in insect cells using baculovirus infection, and isolated using the indicated biotinylated peptides. Bar charts show average and SEM from 5 independent experiments for Cyclin A and BubR1 peptides and 3 independent experiments for Acm1 peptides normalized to the respective wild type peptide. Two-tailed paired t-tests comparing wt and the respective ABBA mutant peptide were used for statistical analysis (**, $p<0.005$; n.s., not significant).

Supplemental Figure 3 – related to Figure 4

Immunoprecipitation experiments from HeLa FRT cell lines stably expressing inducible Flag-mRuby-tagged BubR1 wild-type and mutants. Complexes were immunoprecipitated with an anti-Flag antibody from nocodazole-arrested cells. Average and SEM from 4 independent experiments.

Supplemental Figure 4 – related to Figure 5

(A) The ABBA motif strengthens the SAC in cells with compromised Mps1 activity. Rescue experiments in HeLa FRT cell lines stably expressing inducible siRNA-resistant Flag-mRuby-tagged BubR1, transfected with the indicated siRNA. Cells were synchronised with double thymidine and DMA and AZ3146 were added during the release. Cells were followed by time-lapse microscopy and time spent in mitosis analysed by DIC images. Median values of the time spent in mitosis are shown.

Representative of 3 independent experiments. Unpaired t-test was used for statistical analysis.

(B) Immunofluorescence analysis of HeLa FRT cells stably expressing wild-type or mutant Flag-mRuby-tagged BubR1, transfected with control siRNA or siRNA against BubR1, were treated with taxol. Fixed cells were stained for CDC20 and with CREST serum and the DNA was stained with Hoechst 33342. Scale bars 10 μm.

Supplemental Figure 5 – related to Figure 5 & 6

(A) Western blot analysis of Venus-CDC20 knock in RPE-1 FRT/TO cells. Actin is shown as a loading control.

(B) Still images from a time lapse showing Venus-CDC20 localization in the RPE1 FRT knock-in cells lines. Time is relative to NEBD.

Supplemental Figure 6 – related to Figure 6

(A) Immunofluorescence analysis of HeLa FRT cell lines stably expressing inducible wild-type or mutant Bub1 tagged with Venus-Flag, were transfected with control siRNA or siRNA against Bub1 and treated with taxol. Fixed cells were stained for BubR1 and with CREST serum and the DNA was stained with Hoechst 33342. Scale bar 10 μm.

(B) HeLa cells expressing wild-type or mutant Bub1 tagged with Venus-Flag were treated with taxol and immunoprecipitated using an anti-CDC20. The amount of co-immunoprecipitated BubR1, Bub3 and Mad2 is shown. Average and SEM of at least 3 independent experiments.

(C) HeLa cells treated as in (B) were immunoprecipitated using an anti-APC4 antibody. The amount of co-immunoprecipitated CDC20, BubR1 and Mad2 is shown. Average and SEM of at least 3 independent experiments.

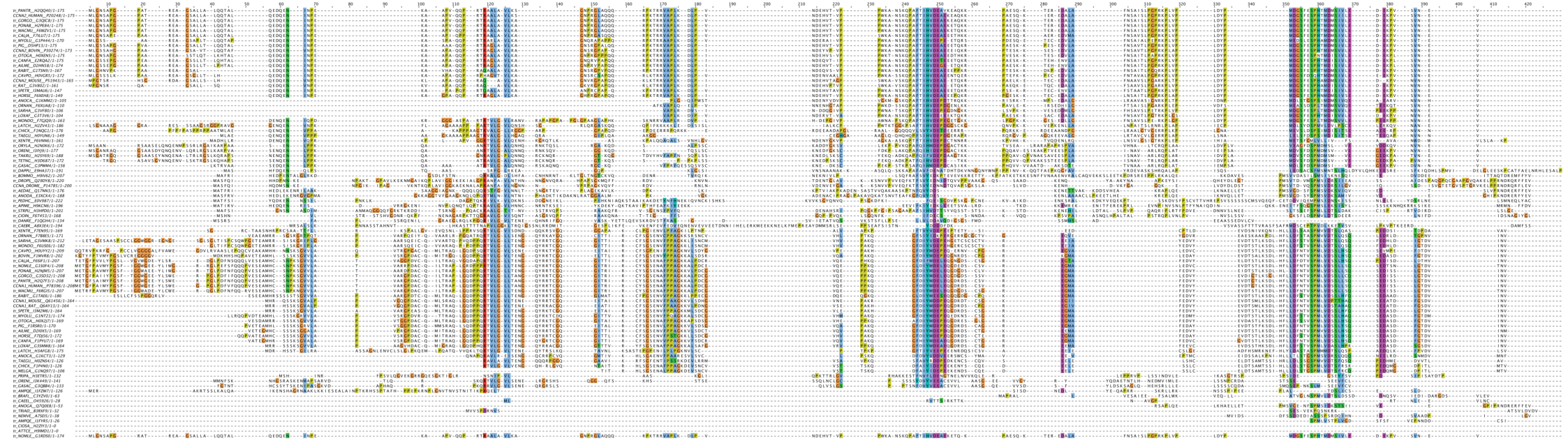
Supplemental Table 1 – related to Figure 1

List of proteins used for the SLiMPrints analysis. Only reviewed human proteins from the UniProt database annotated with the GO term "mitosis" were considered.

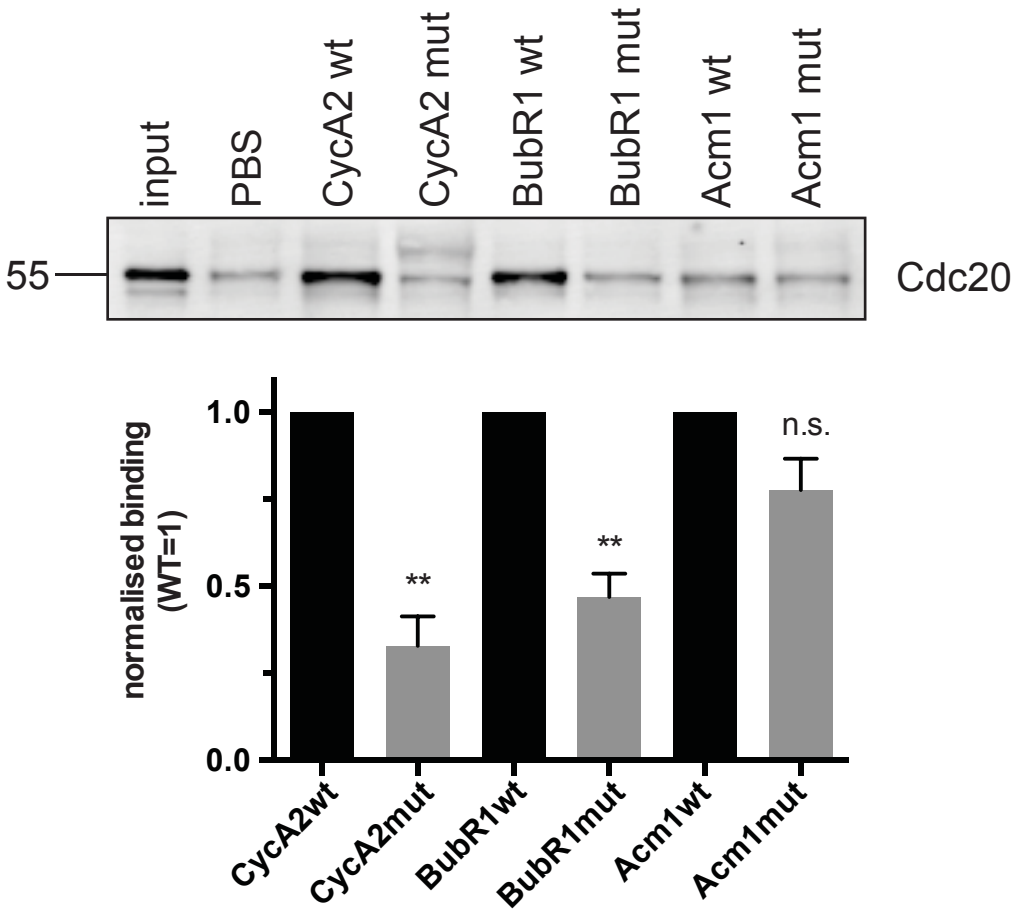
Supplemental Table 2 – related to Figure 1

A: List of significant motif hits (sig > 0.0001) from a SLiMPrints analysis of reviewed human proteins from the UniProt database annotated with the GO term "mitosis". Returned instances were filtered for accessibility by limiting the search to predicted disordered regions and filtering out extracellular and transmembrane regions, and annotated globular domains

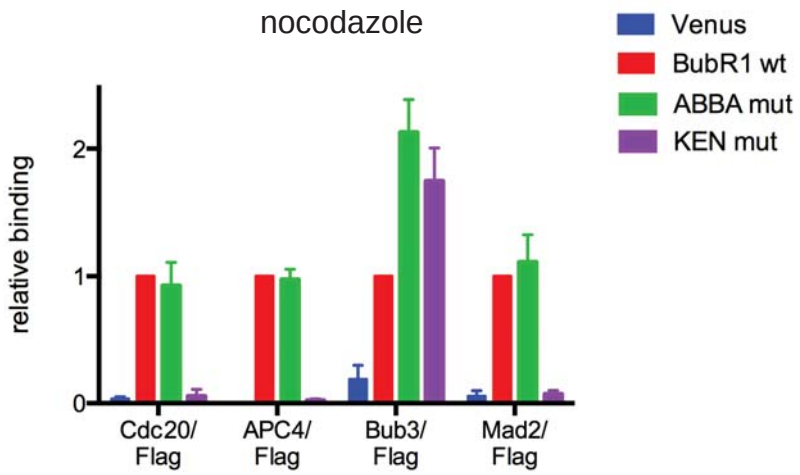
B: List of motif hits in all reviewed human proteins from the UniProt database from a SLiMSearch analysis with the regular expression Fx[ILV][FHY]x[DE]. Returned instances were filtered for accessibility by limiting the search to predicted disordered regions and filtering out extracellular and transmembrane regions, and annotated globular domains



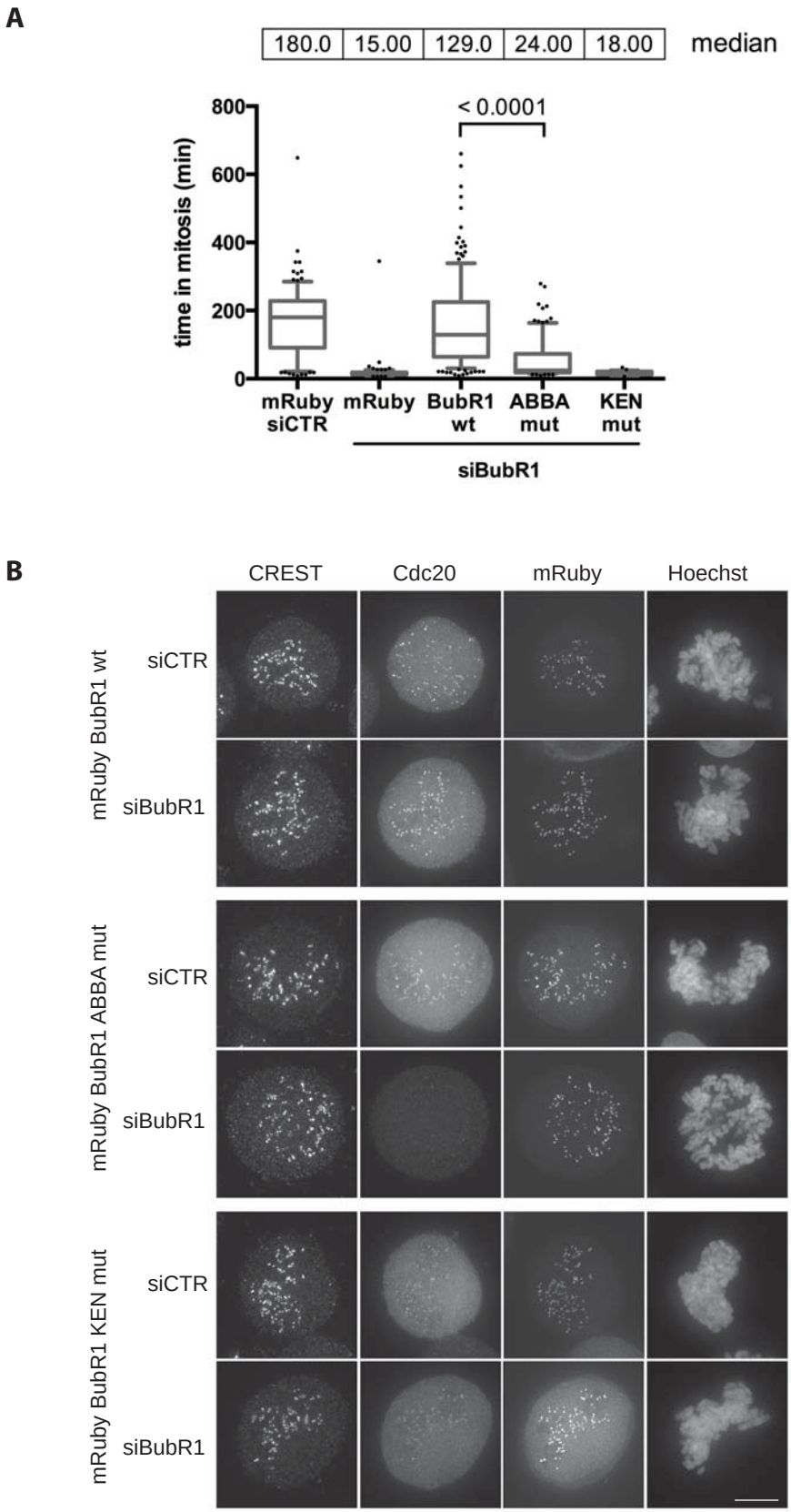
Di Fiore et al, supplemental figure 1



Di Fiore et al, Supplemental figure 2

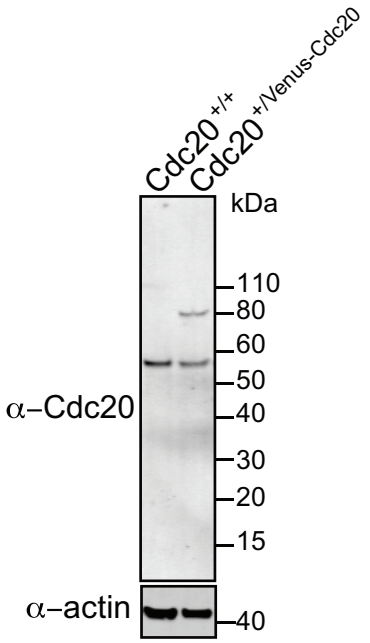


Di Fiore et al, Supplemental figure 3

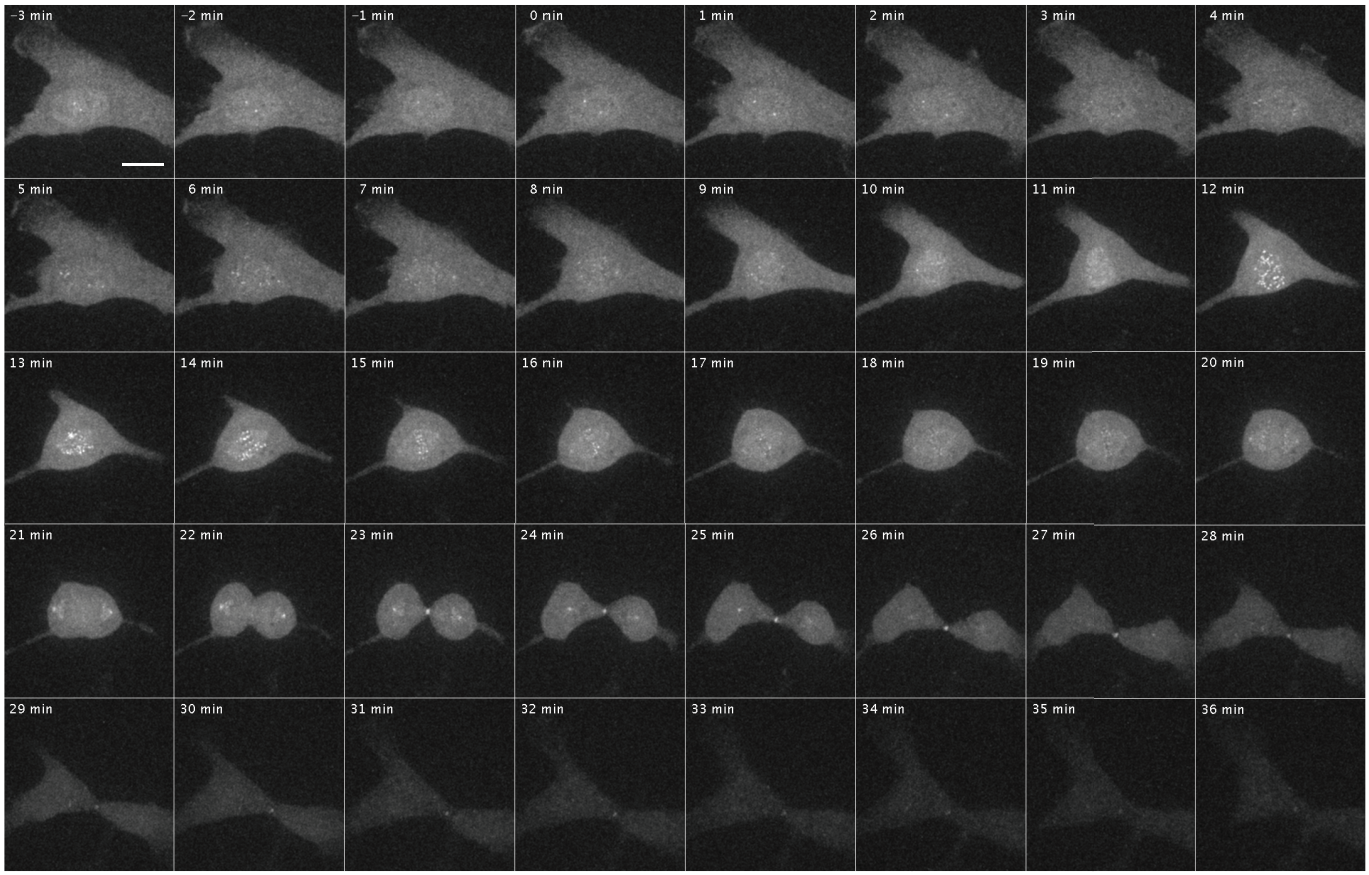


Di Fiore et al, Supplemental figure 4

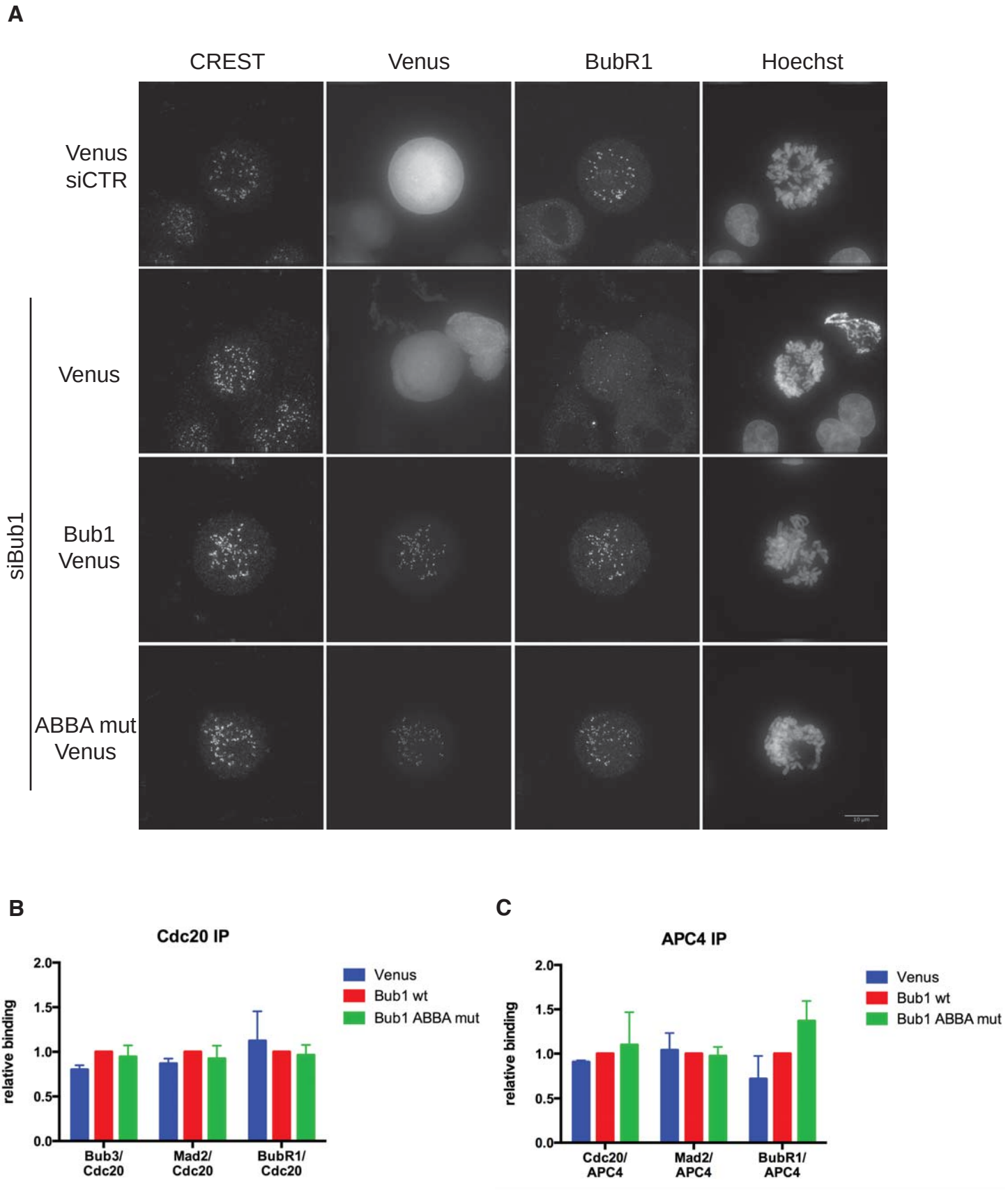
A



B



Di Fiore et al, Supplemental figure 5



Di Fiore et al, Supplemental figure 6