



Figure S3. **Dynactin-depleted cells do not retain checkpoint proteins at their kinetochores.** (A) HeLa cells were transfected with indicated siRNAs for 72 h. 5 h before fixation, cells were treated with the CDK1 inhibitor RO3306. After 3 h, cells were released from the RO3306 block by washing four times with PBS to allow cells to go in mitosis. One sample was treated with nocodazole to create unattached KTs. After 2 h, cells were fixed and stained with BUBR1 antibody and with CREST antibody to visualize the centromeres. (B) HeLa cells were treated as in A. Cells were stained for MAD1 and CREST. (C) HeLa cells were treated as in A. Cells were stained for CDC20 and CREST. (D) HeLa cells were treated as in A. Cells were stained for Spindly and CREST. Insets are enlargements of individual KT pairs. Bars, 5 μm (insets, 0.5 μm).

