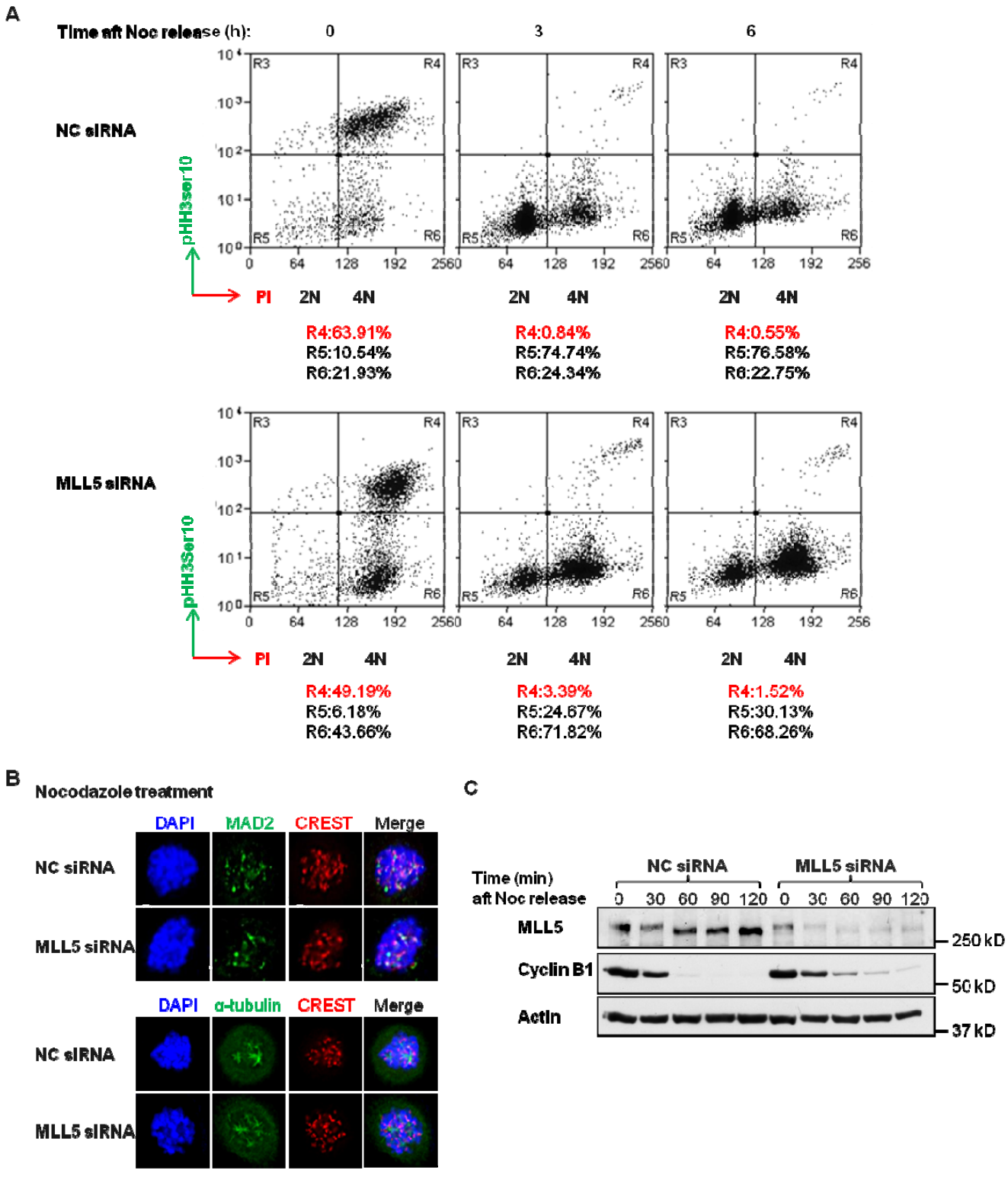


Fig. S1. (A) MLL5-depleted mitotic cells exited from mitosis without proper DNA segregation. U2OS cells were transfected with NC- or MLL5-siRNA and synchronized to M phase by nocodazole. Mitotic cells were collected and released in nocodazole-free medium and harvested at indicated time intervals. The cells were stained with PI and anti-phospho-histone H3Ser10 antibody, and analyzed by flow cytometry. Noc, nocodazole. R4 region is gated on mitotic cells, and the number indicates the percentage of cells remained at mitosis. (B) The activation of mitotic checkpoint was unaffected by MLL5 knockdown. U2OS cells were transfected with NC- or MLL5-siRNA for 24 h, and subsequently incubated with nocodazole or taxol for 16 h. Cells were stained with MAD2 (green), CREST (red) and α-tubulin (green) antibodies. DNA was counterstained with DAPI. Scale bar, 5 μm. α-Tubulin serves as a control for drug efficiency in the lower panel. (C) The activity of the anaphase-promoting complex/cyclosome (APC/C) was un-affected by MLL5 depletion. Cell extracts were prepared after mitotic release, and the expressions of MLL5 and cyclin B1 were studied by western blotting. Actin served as a loading control. Numbers next to gel blots indicate molecular mass in kilodaltons.

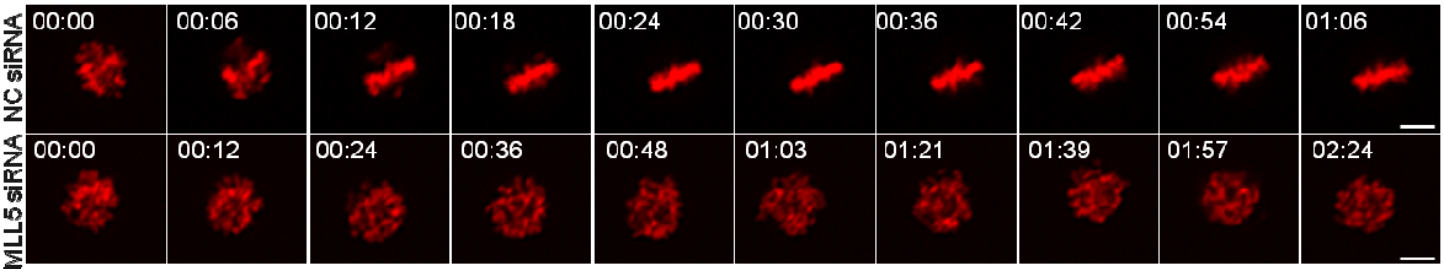


Fig. S2. The chromosome alignment process for the control and MLL5-depleted cells was monitored by live-cell imaging. U2OS cells were transfected with NC- or MLL5-siRNA and synchronized to M phase by nocodazole. Mitotic cells were collected and released in medium containing MG132 to prevent anaphase onset, and the chromosome alignment process was live-imaged. MLL5-depleted cells failed to align their chromosomes at metaplate even at 2 h after nocodazole release, while control cells almost reached complete alignment after only 12 min of release. Scale bar, 10 μm.

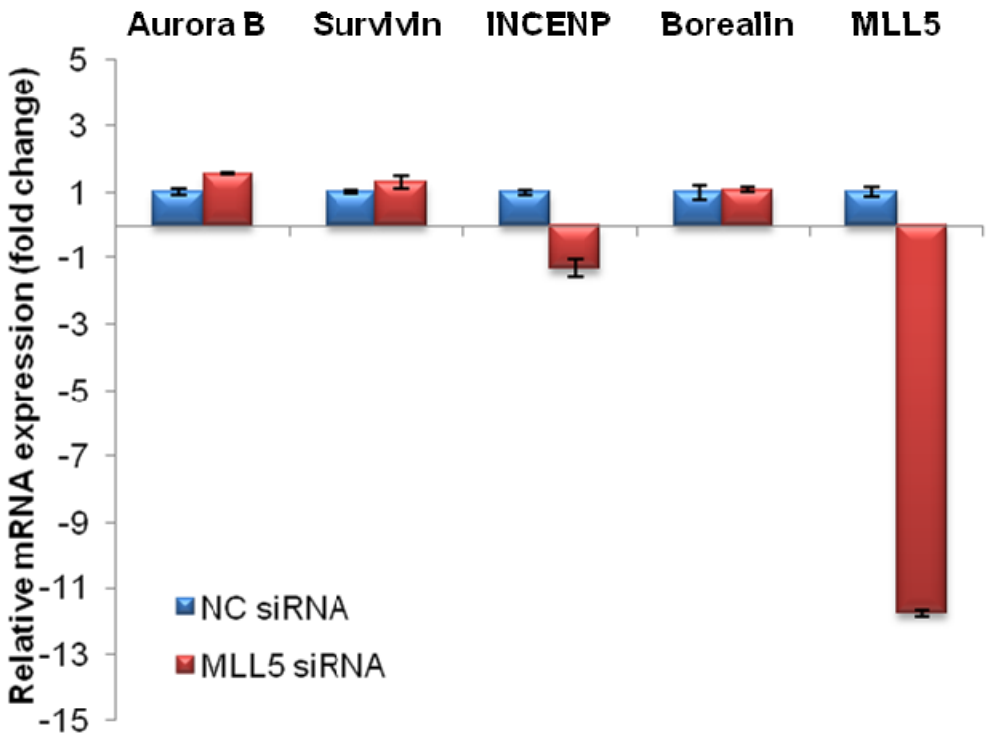
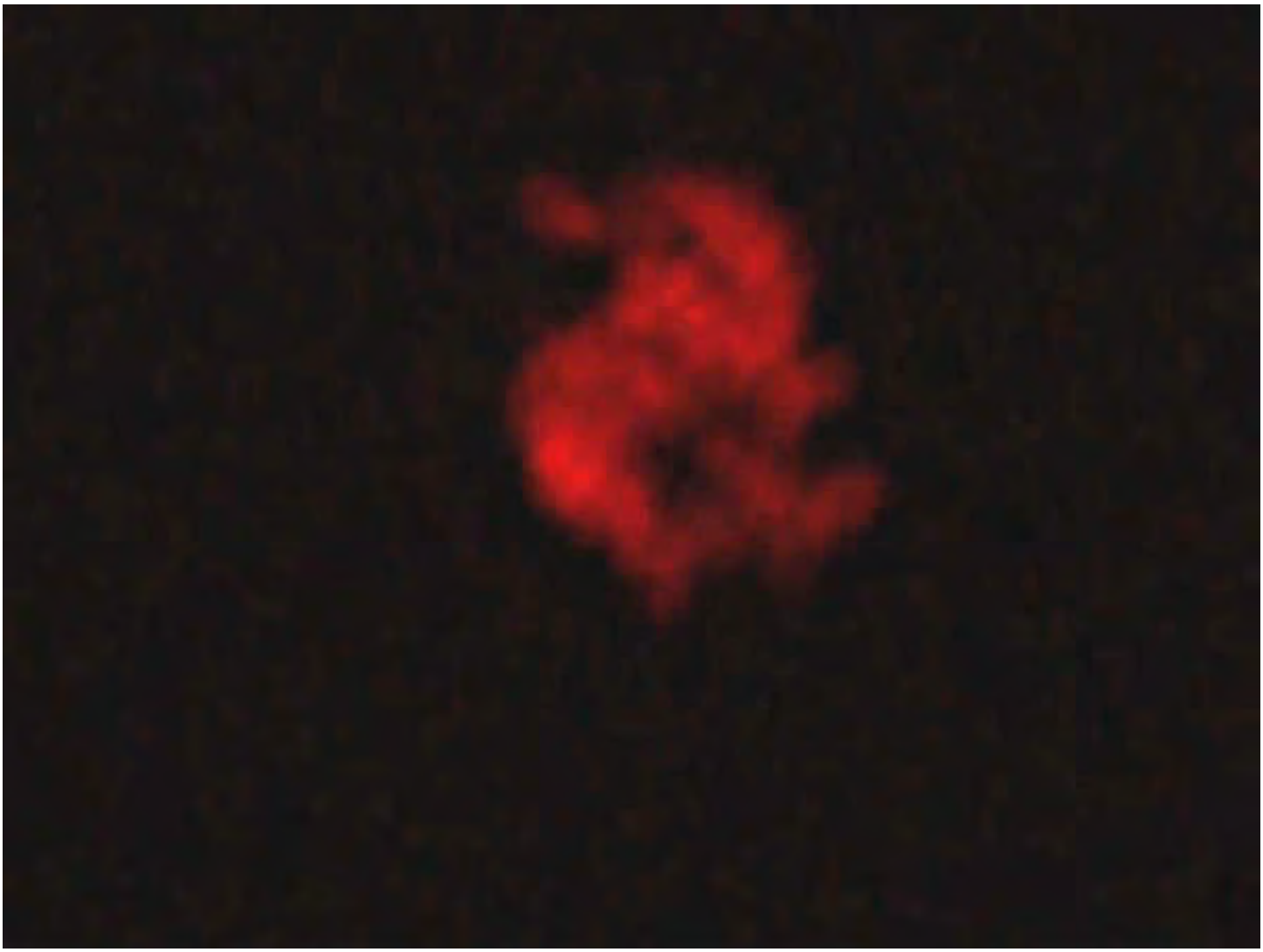
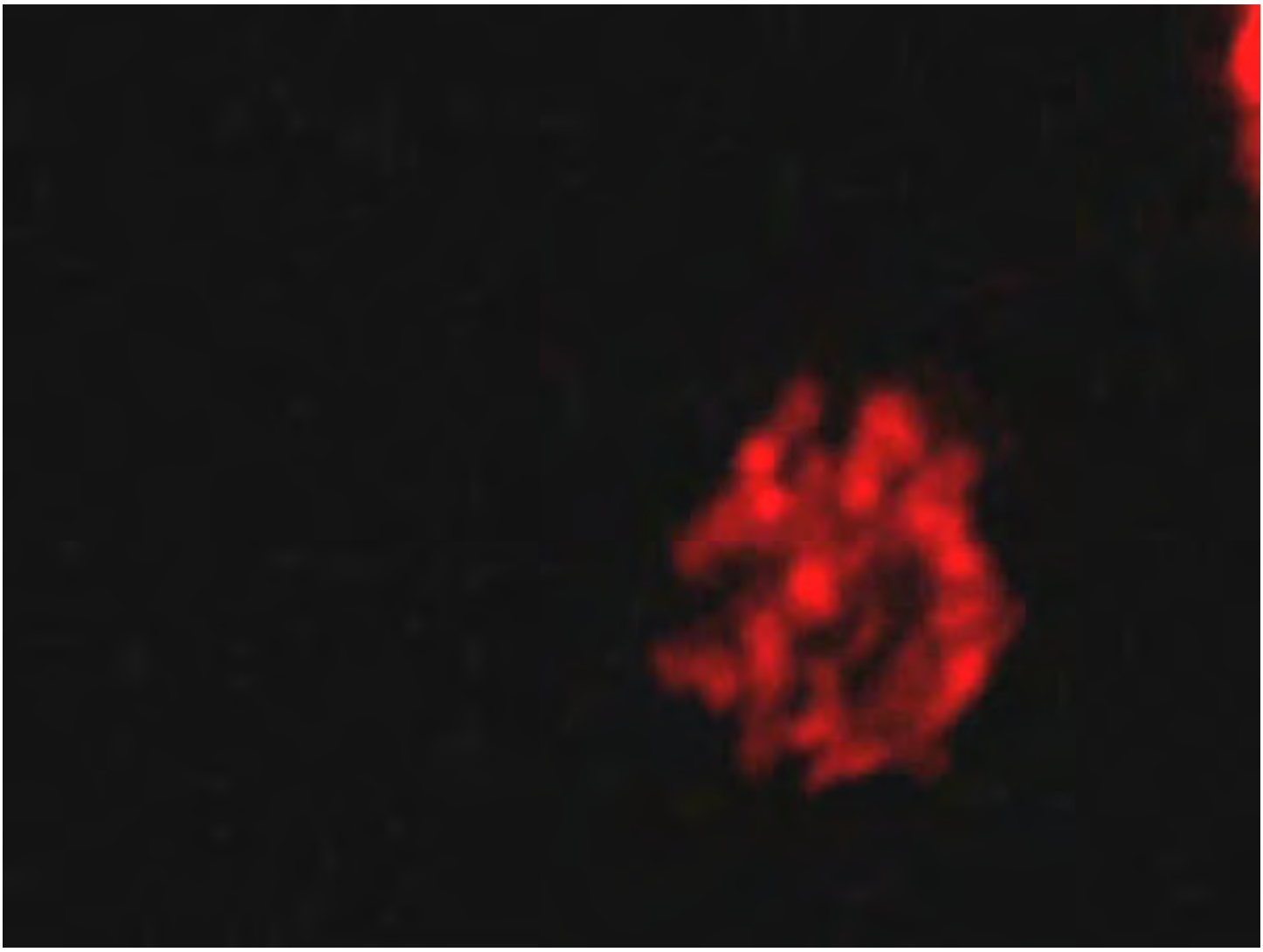


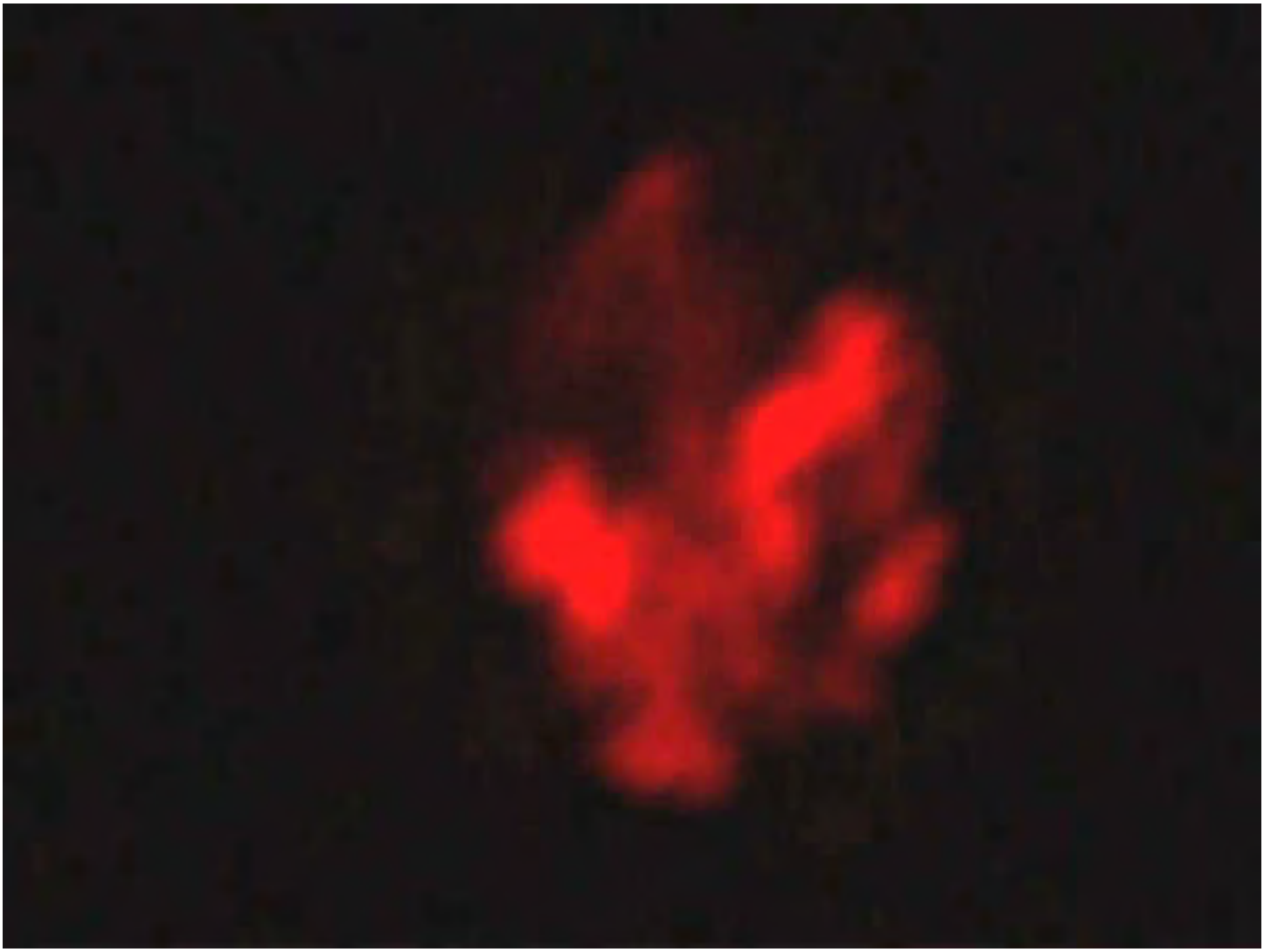
Fig. S3. Quantitative RT-PCR study showed that the mRNA level of CPC components after MLL5-knockdown was not significantly affected. U2OS cells were transfected with NC- or MLL5-siRNA and synchronized to M phase by nocodazole. Total RNA was extracted using TRIzol reagent and used as a template for real-time RT-PCR reaction. The real-time RT-PCR reaction was performed using the iScriptTM One-Step RT-PCR Kit with SYBR®Green. The mRNA abundance of the Aurora B, Survivin, INCENP, Borealin and MLL5 in MLL5-depleted cells was normalized to the mRNA abundance of these genes in NC-siRNA control cells. RPL13A was used as an internal control.



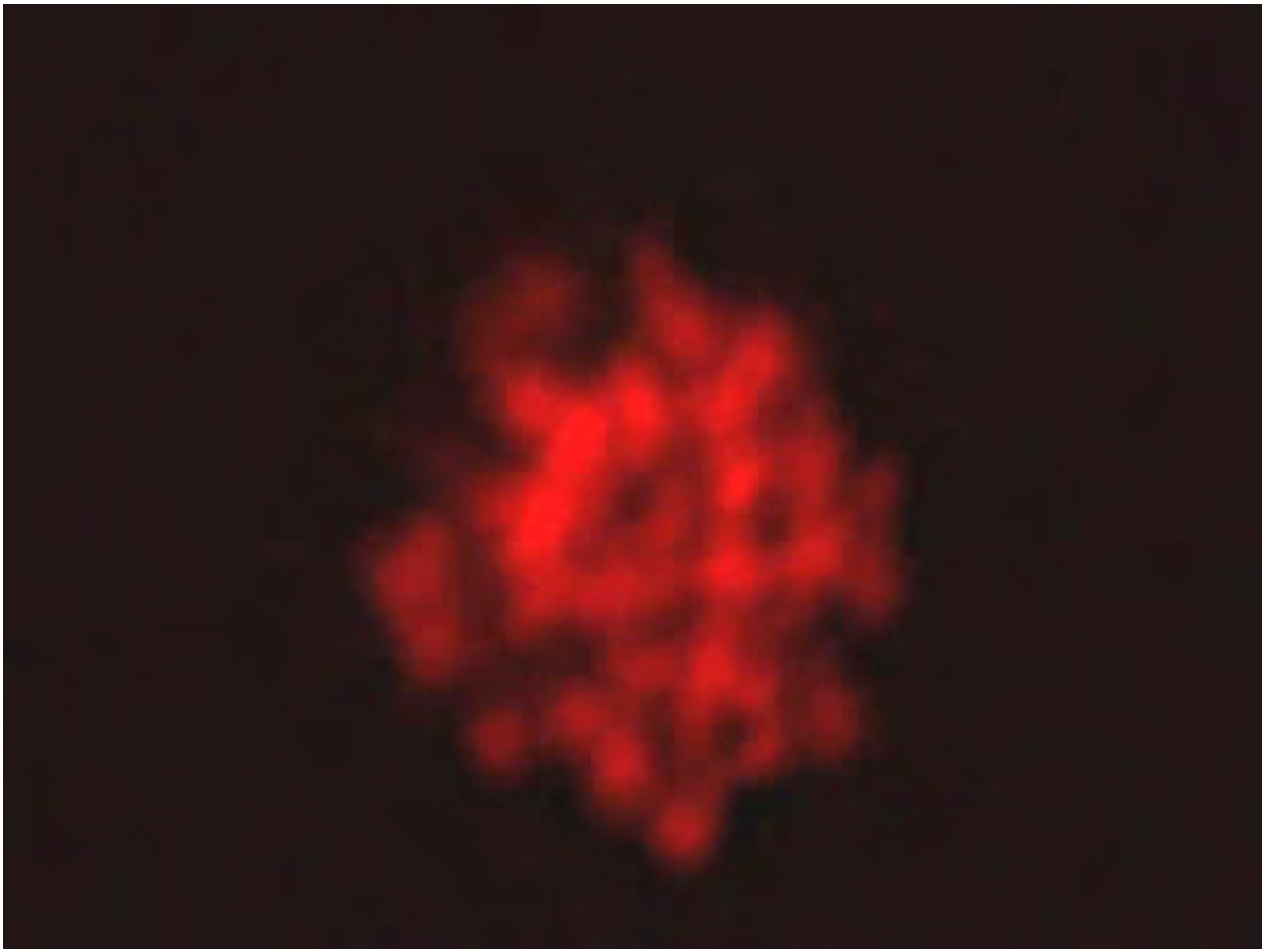
Movie.1. Mitotic progression of NC-siRNA cells.



Movie 2. Mitotic progression of MLL5-siRNA cells.



Movie 3. Chromosome alignment at metaphase in NC-siRNA cells.



Movie 4. Improper chromosome alignment at metaphase in MLL5-siRNA cells.