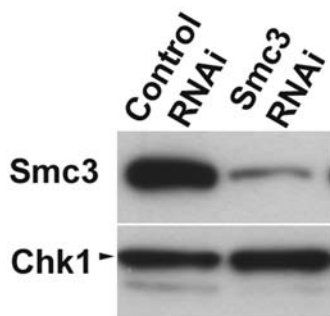
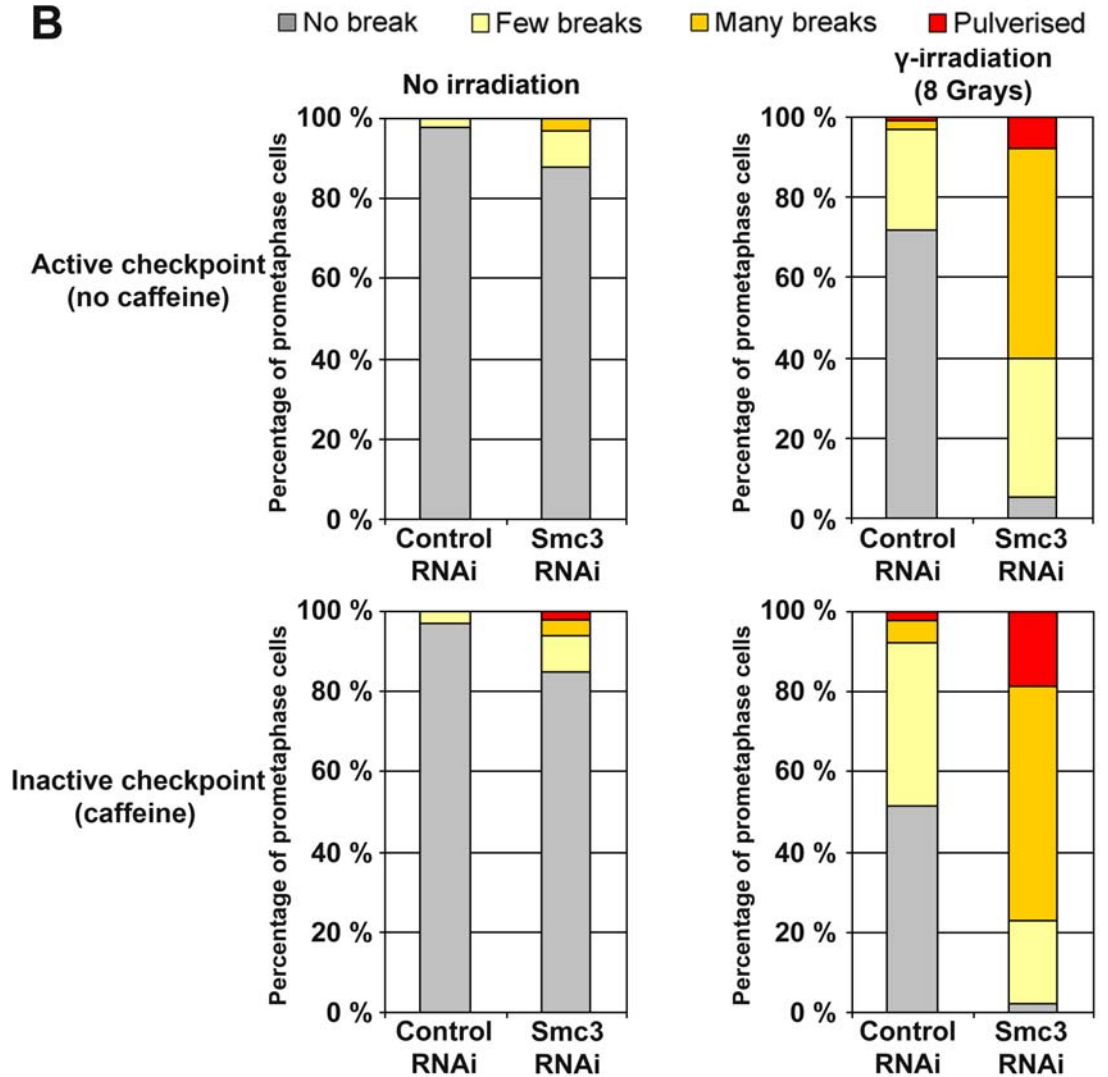


Watrin_Supplemental Figure 1

A

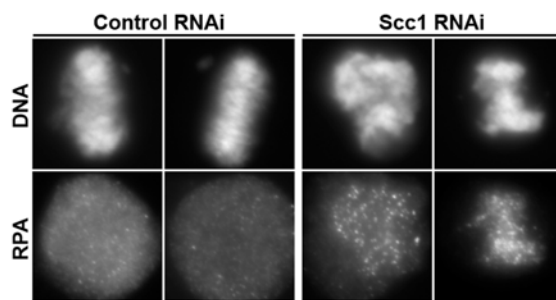


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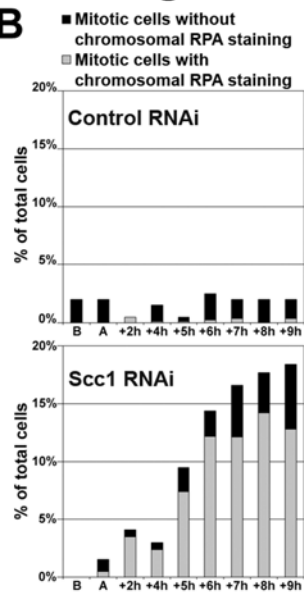


Watrin_Supplemental Figure 2

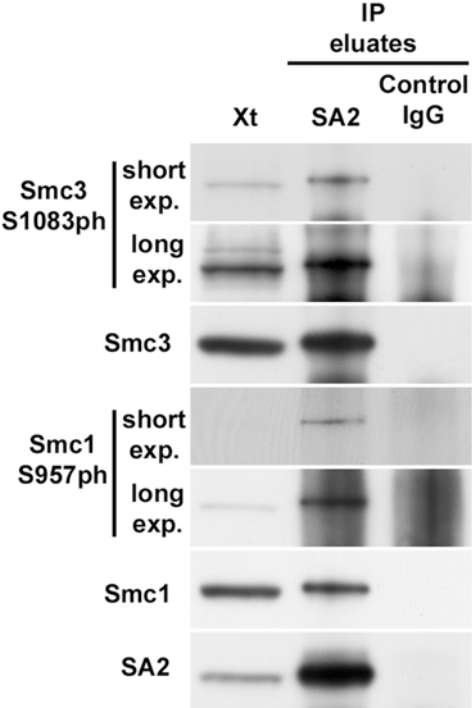
A



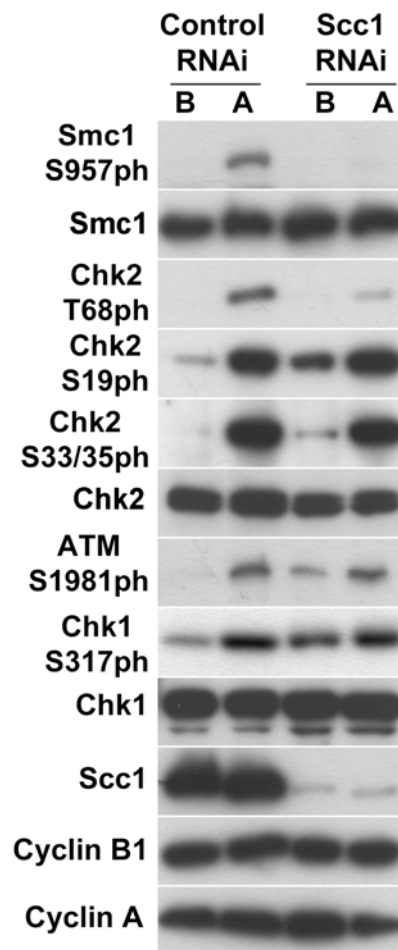
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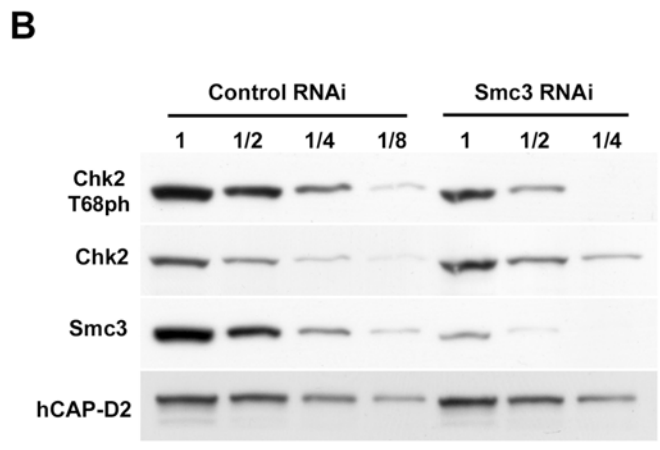
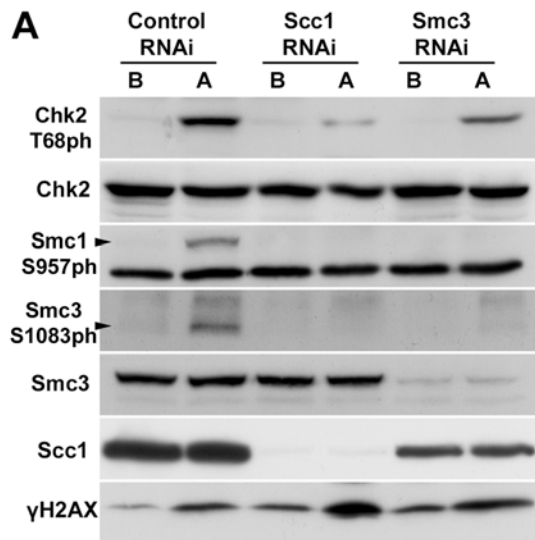
Watrin_Supplemental Figure 3



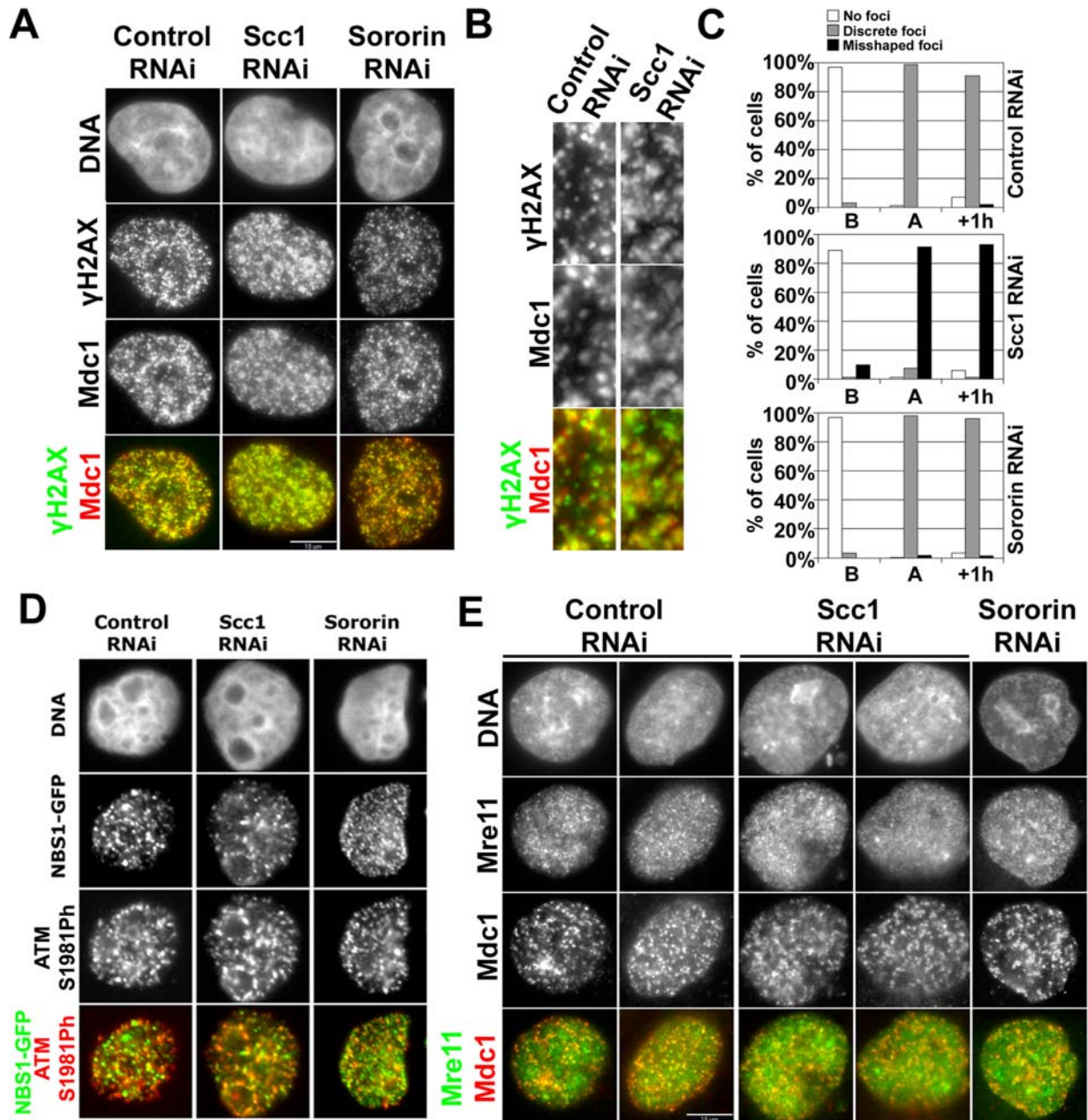
Watrin_Supplemental Figure 4



Watrin_Supplemental Figure 5

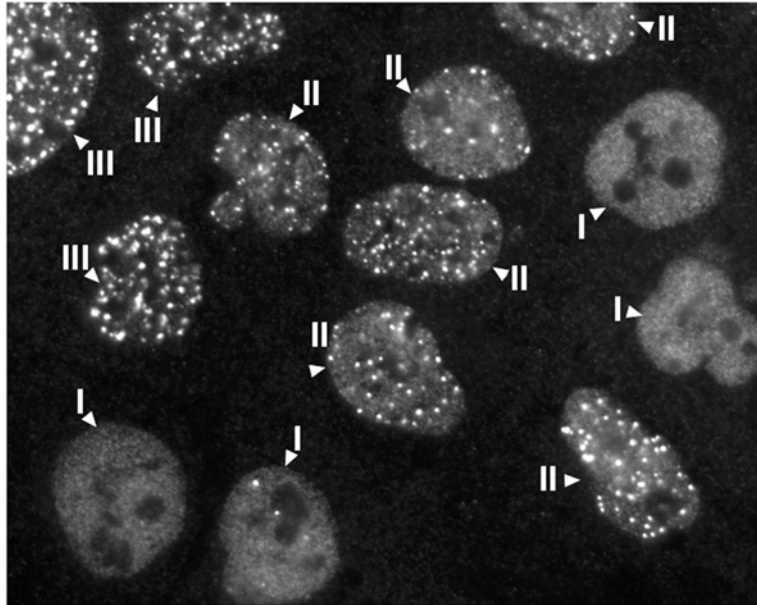


Watrin_Supplemental Figure 6

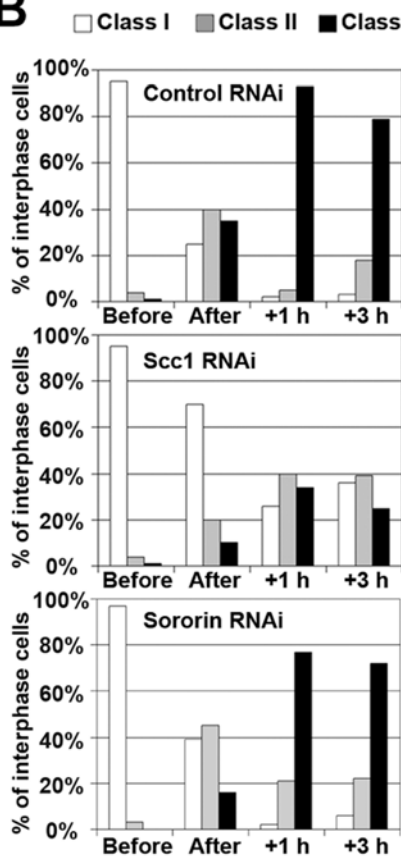


Watrin_Supplemental Figure 7

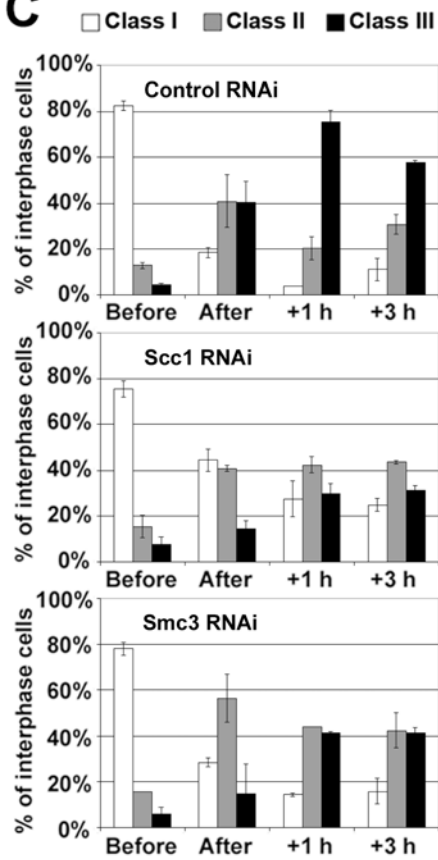
A



B



C



Supplemental Figure legends

Supplemental Figure 1. Smc3 depleted cells enter mitosis despite unrepaired DNA DSBs.

HeLa cells were synchronised by double thymidine arrest and were transfected with siRNAs during the first arrest. 6 hours after release from the second arrest, cells were irradiated (8 Grays). One hour after recovery, cells were eventually treated with 2 mM caffeine, and arrested in mitosis by 0.1 μ M nocodazole. 10 hours later, mitotic chromosomes were spread and stained with Giemsa.

(A) Western blot analysis of control and Smc3 depleted HeLa cells.

(B) Quantification of double strand break phenotypes (described in Figure 1) observed in the experiment depicted in (A) ($n \geq 100$ per condition).

Supplemental Figure 2. Scc1 depleted cells that prematurely entered mitosis retain chromosomal RPA staining.

Control, Scc1 and sororin depleted cells were irradiated in G2 phase. At the indicated times, cells were fixed with 1:1 methanol/acetone mixture, stained with DAPI and processed for immunofluorescence using anti-RPA antibodies.

(A) Representative fluorescence microscopy pictures of mitotic cells from control and Scc1 depleted cells.

(B) Mitotic cells exhibiting chromosomal RPA staining were counted and expressed as a percentage of total cells ($n > 100$).

Supplemental Figure 3. Phosphorylated Smc1 at S957 and Smc3 at S1083 are specifically co-immunoprecipitated with the cohesin subunit SA2.

HeLa cell extract was prepared after treatment with etoposide (5 μ M for 30 minutes), and incubated with antibodies specific for the cohesin subunit SA2 (SA2) or with control IgG (control IgG). Extract (Xt) and immunoprecipitates (IP eluates) were analysed by Western blotting using the indicated antibodies (exp.: exposure).

Supplemental Figure 4. Cohesin is specifically required for Chk2 phosphorylation at T68.

Synchronised control and Scc1 depleted cells were irradiated (8 Grays) in G2 phase, and total cell extracts were prepared prior (B) and soon after (A) irradiation, and analysed by Western blotting using the indicated antibodies.

Supplemental Figure 5. Full activation on Chk2 upon DNA damage depends on cohesin

(A) Control Scc1 and Smc3 depleted cells were synchronised in G2 phase and were treated with etoposide (5 μ M for 15 minutes). Total cell extracts were prepared prior (B) and soon after (A) DNA damage induction and analysed by Western blotting using the indicated antibodies.

(B) Dilution series of irradiated control cells and Smc3 depleted cells were analysed by Western blotting using the indicated antibodies.

Supplemental Figure 6. Scc1 depletion does not affect enrichment of Mdc1, γ H2AX, ATM-S1981Ph, Mre11 and Nbs1 at IRIFs but alters IRIF structure.

(A) Control, Scc1 and Sororin depleted cells were irradiated in G2 phase and processed for immunofluorescence experiments using Mdc1 and γ H2AX antibodies. Representative pictures are shown. Phenotypes are described in (C).

(B) Enlargement of pictures shown in (A) highlighting the effect of Scc1 depletion on IRIF structure as judged by Mdc1 and γ H2AX staining.

(C) Quantification of IRIF structure phenotypes, which were classified as follow: homogenous nuclear staining (no foci, typically observed before DNA damage induction); well-defined IRIFs (discrete foci) observed in control and sororin depleted cells after irradiation; IRIFs that appear larger and more diffuse (misshaped foci) than that of control cells. Numbers shown were obtained from one experiment representative of three independent experiments.

(D) Representative fluorescence microscopy pictures of control, Scc1 and sororin depleted U2OS cells expressing Nbs1-GFP irradiated in G2 phase (8 Grays) and processed for immunofluorescence (ATM-S1981Ph) shortly after irradiation.

(E) Control, Scc1 and Sororin depleted cells were irradiated in G2 phase and processed for immunofluorescence experiments using Mdc1 and Mre11 antibodies. Representative pictures are shown.

Supplemental Figure 7. Cohesin is required for the proper recruitment of 53BP1 to DNA damage site.

Control, Scc1, Sororin (B) and Smc3 (C) depleted HeLa cells were irradiated (8 Grays) in G2 phase, processed for immunofluorescence using 53BP1 antibody and counterstained with DAPI. Cells were classified as in Figure 6 according to 53BP1 pattern in three different classes: homogenous nuclear staining (class I), few foci with diffuse staining (class II) and many strong foci without diffuse staining (class III).

(A) Representative fluorescence microscopy pictures of Scc1 depleted cells fixed three hours after irradiation illustrating the three classes (I, II and III) of 53BP1 staining.

(B) Quantification of the phenotypes before, soon after, one hour (+1h) and three hours (+3h) after irradiation for control, Scc1 and sororin depleted cells.

(C) Quantification of the phenotypes before, soon after, one hour (+1h) and three hours (+3h) after irradiation for control, Scc1 and Smc3 depleted cells. Averages and standard deviations calculated from two independent experiments are shown.