

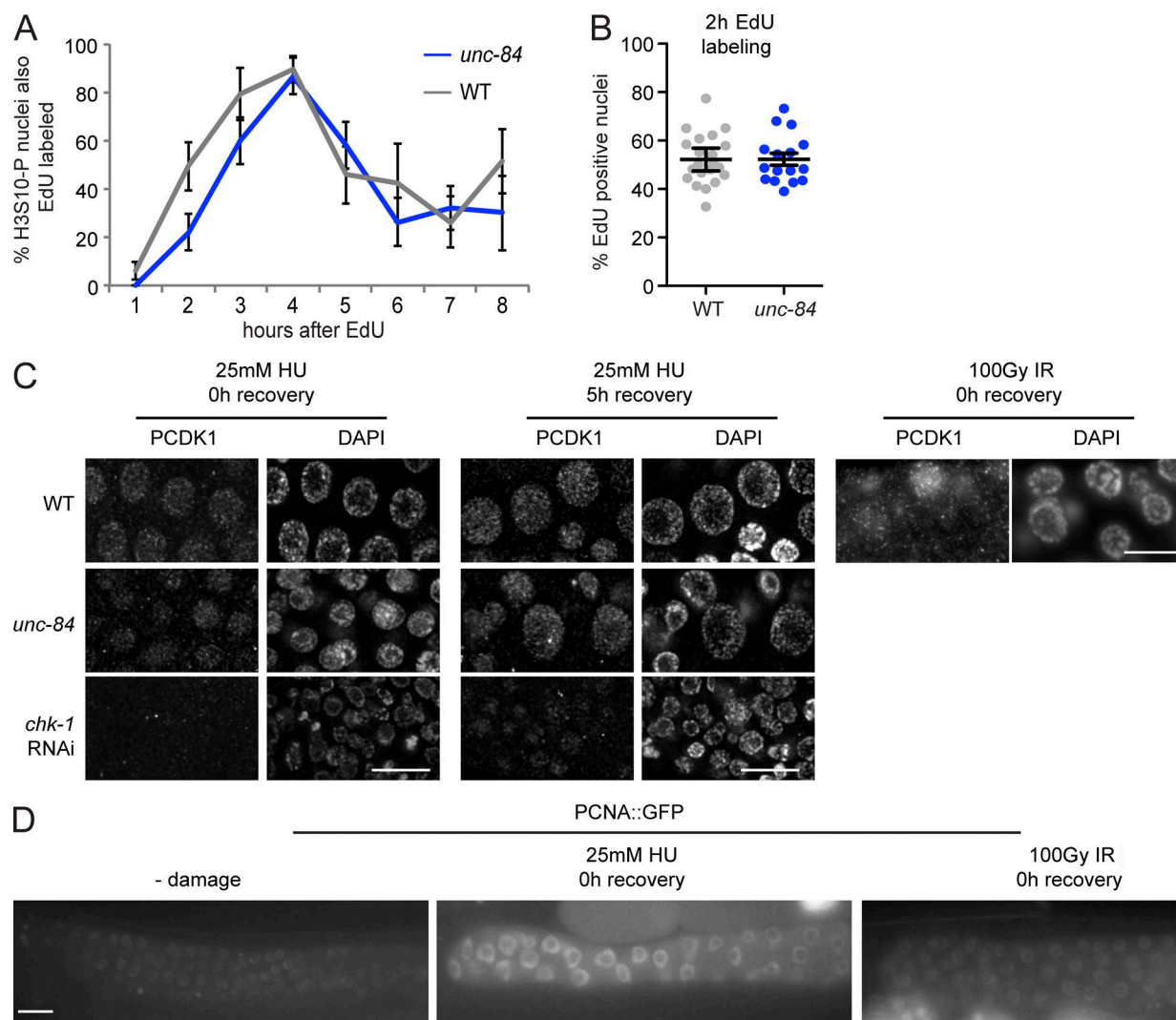
Lawrence et al., <https://doi.org/10.1083/jcb.201604112>

Figure S1. **Cell cycle effects.** (A) Cell cycle kinetics were monitored by pulse-chase after 30 min of EdU labeling and staining for H3S10-P, a marker of prometaphase/metaphase (Fox et al., 2011); graph shows the percentages of H3S10-P-positive nuclei that are also EdU positive in wild type (WT) and *unc-84(n369)* over time. Error bars indicate 95% CI between two biological replicates. $n > 30$. (B) Percentages of EdU-positive nuclei after 2 h of continuous labeling in wild type and *unc-84(n369)*. Error bars represent 95% CI. (C) Phospho-CDK-1/NCC-1 (PCDK-1), a marker of G2 (Moser et al., 2009) after treatment with HU or IR. (D) PCNA::GFP, a marker of S phase (Kisielewska et al., 2005), in the absence or presence of HU or IR. Bars, 10 μ m.

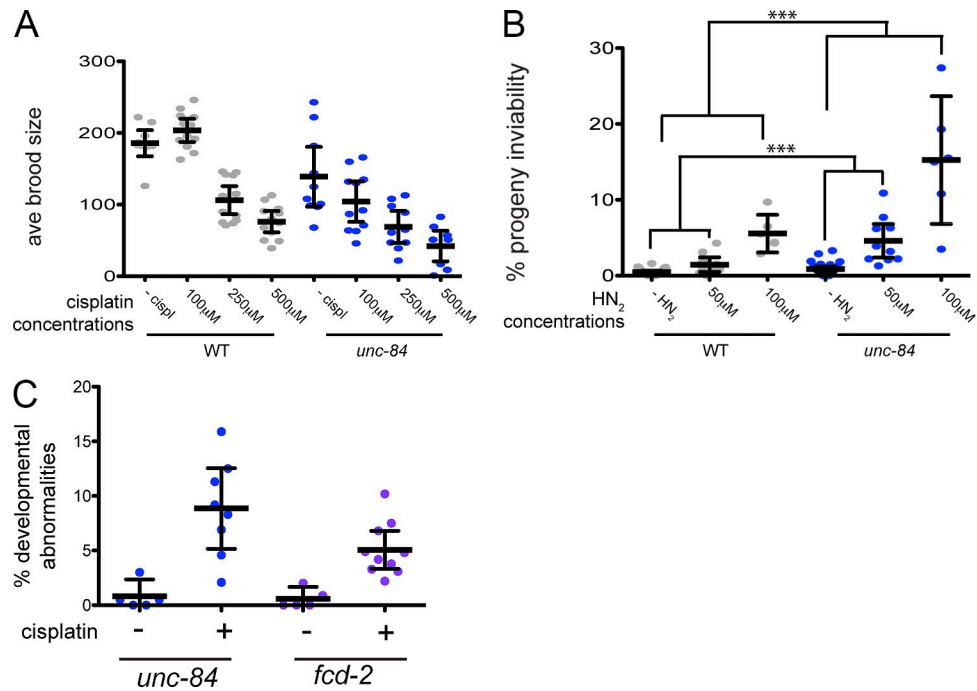


Figure S2. **Cross-linking agents reduce *unc-84(n369)* brood size and progeny viability and increase developmental abnormalities.** (A) Brood size after 0, 100, 250, or 500 μ M cisplatin in wild-type (WT) and *unc-84(n369)* germlines. $n > 10$. cispl, cisplatin. (B) Progeny inviability after 0, 50, or 100 μ M of nitrogen mustard in wild-type and *unc-84(n369)* germlines. $n > 10$. (C) Frequency of postembryonic developmental morphological abnormalities (larval arrest, vulva defects, and retarded growth) in the indicated genotypes observed before and after treatment with cisplatin. Error bars indicate 95% CI. P-values were determined by two-way analyses of variance. ***, $P < 0.0001$.

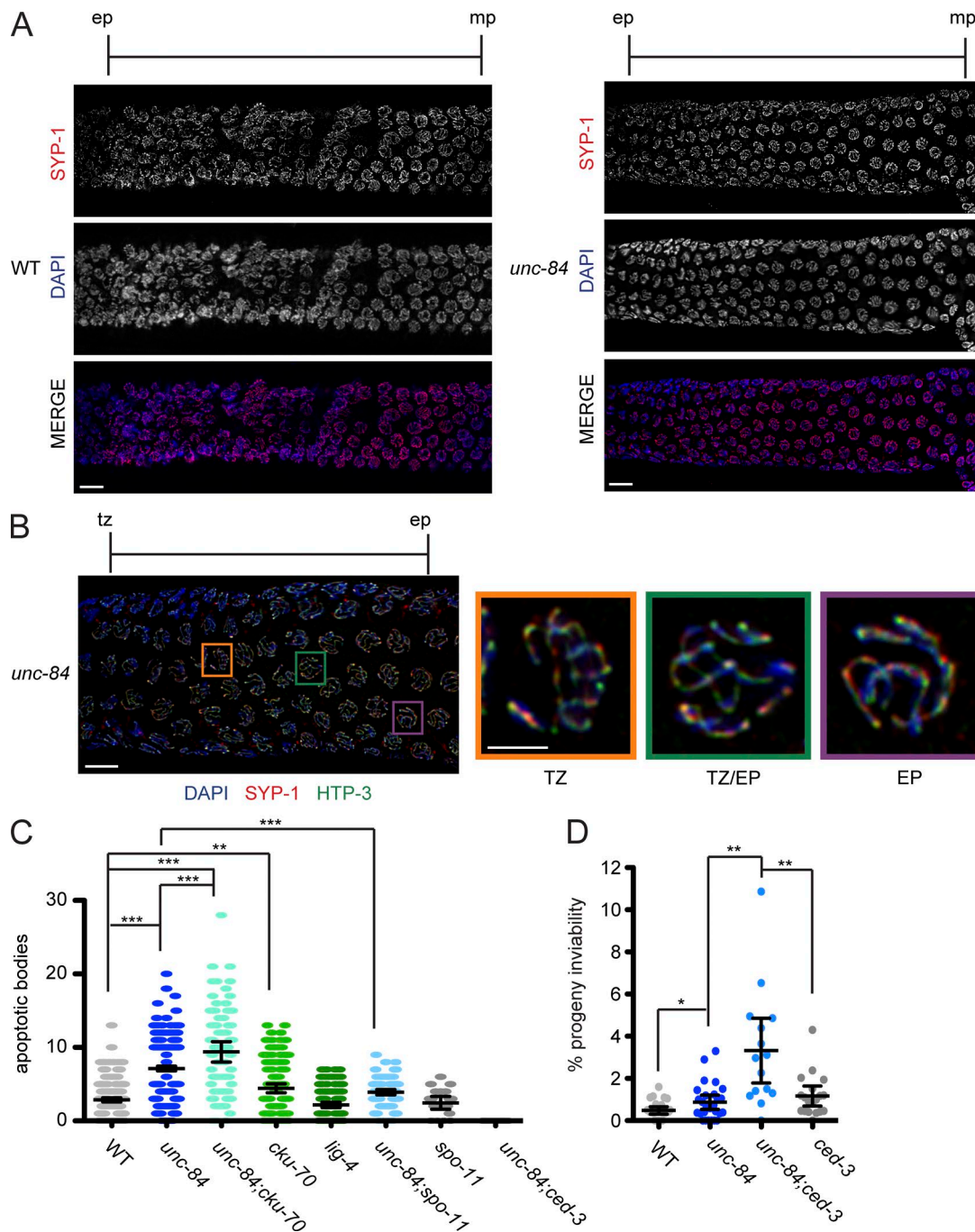


Figure S3. *unc-84* mutants do not display defects in chromosome synapsis but have elevated germline apoptosis. (A) Wild-type (WT) and *unc-84(n369)*-dissected germlines stained with antibodies against SYP-1 (red) and DAPI (blue). In both genotypes, SYP-1 loads after transition zone in the early pachytene (ep) and remains associated with chromatin through the late pachytene. mp, mid-pachytene. (B) Proper SYP-1 loading in *unc-84(n369)* was also determined by confirming the association of SYP-1 (red) tracks with HTP-3 (green), an axial component, in the transition zone (TZ) and early pachytene (EP). (C) Number of apoptotic bodies visualized with AO 48 h after L4 in wild type (N2), *unc-84(n369)*, *unc-84(n369);cku-70(tm1524)*, *cku-70(tm1524)*, *lig-4(ok716)*, *unc-84(n369);spo-11(ok79)*, *spo-11(ok79)*, and *unc-84(n369);ced-3(n717)*. (D) Percentages of progeny inviability in wild type (N2), *unc-84(n369)*, *unc-84(n369);ced-3(n717)*, and *ced-3(n717)*. Bars: (main images) 10 μ m; (insets) 2 μ m. Error bars indicate 95% CI. *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$.

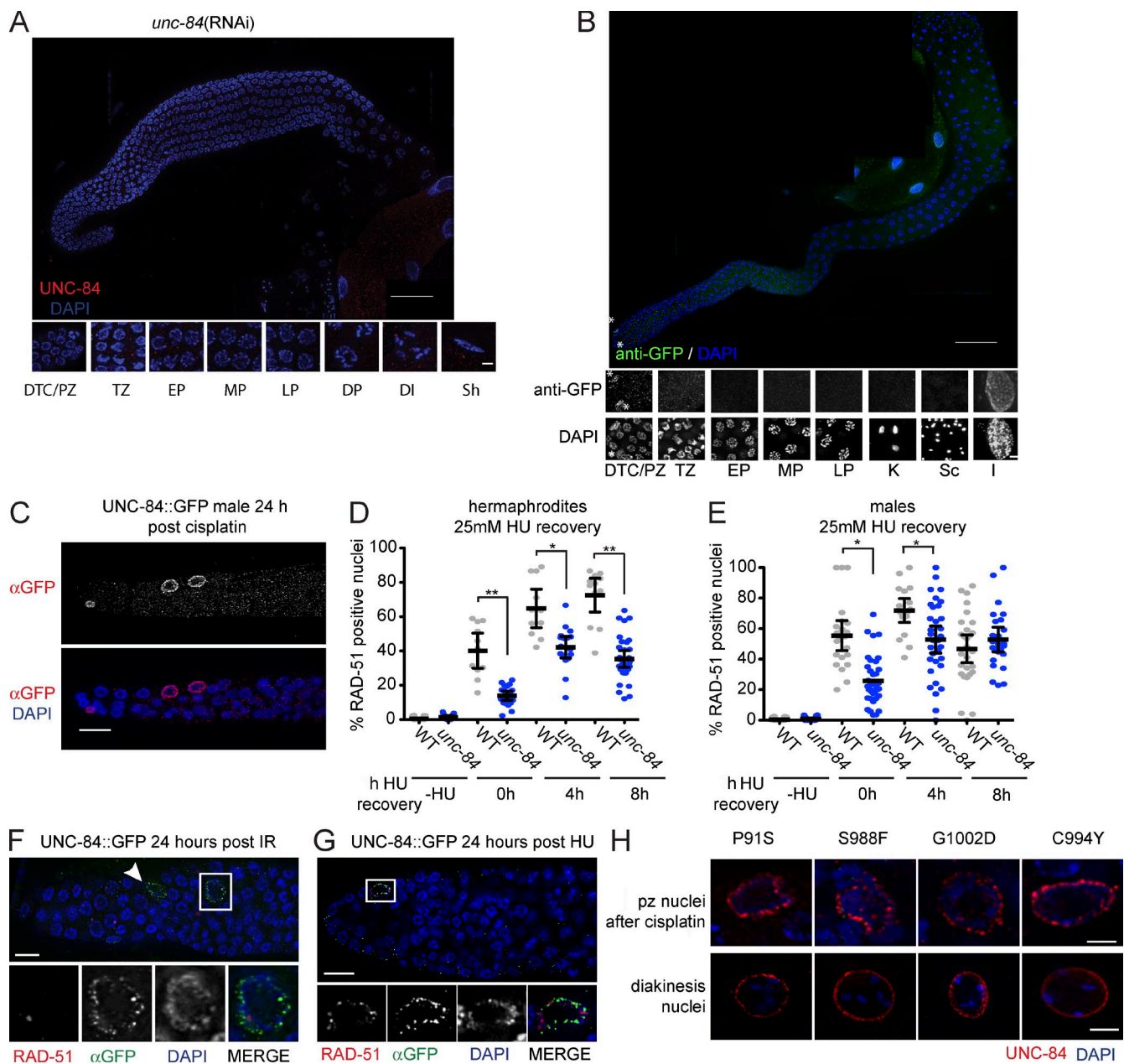


Figure S4. UNC-84::GFP in male germlines and in response to different DNA-damaging agents. (A) *unc-84(RNAi)* in the UNC-84::GFP strain confirms anti-GFP is recognizing UNC-84. (B) UNC-84::GFP-dissected germline stained for anti-GFP (green) and DAPI (blue). UNC-84 does not localize to late pachytene nuclei in the male germline. (A and B) Insets show staining in all zones of the germline from the PZ, transition zone (TZ), early pachytene (EP), mid-pachytene (MP), late pachytene (LP), diplotene (DP), diakinesis (DI), and karyosome (K), as well as the somatic distal tip cell (DTC), sheath cell (Sh), and intestinal cells (I). Anti-GFP staining is only evident in somatic distal tip cells and intestinal cells. Bars: (main images) 100 μ m; (insets) 2 μ m. (C) UNC-84 is expressed in male PZ germ cells after cisplatin recovery. (D) Percentages of RAD-51-positive nuclei after release from 25 mM HU over the given times in wild-type (WT; N2) and *unc-84(n369)* hermaphrodites. $n > 12$. (E) Percentages of RAD-51-positive nuclei in *unc-84(n369)* male germlines after recovery from 25 mM HU. DNA repair defects in *unc-84(n369)* are not unique to hermaphrodites. $n > 15$. (F and G) UNC-84 is expressed in PZ germ cells after HU recovery (F) and IR recovery (G). The arrowhead in F marks another GFP-positive nucleus. (H) UNC-84 localizes to the nuclear envelope of PZ nuclei after damage and diakinesis nuclei in UNC-84 point mutants. Bars, 10 μ m. P-values were obtained by t test. *, $P < 0.01$; **, $P < 0.001$.

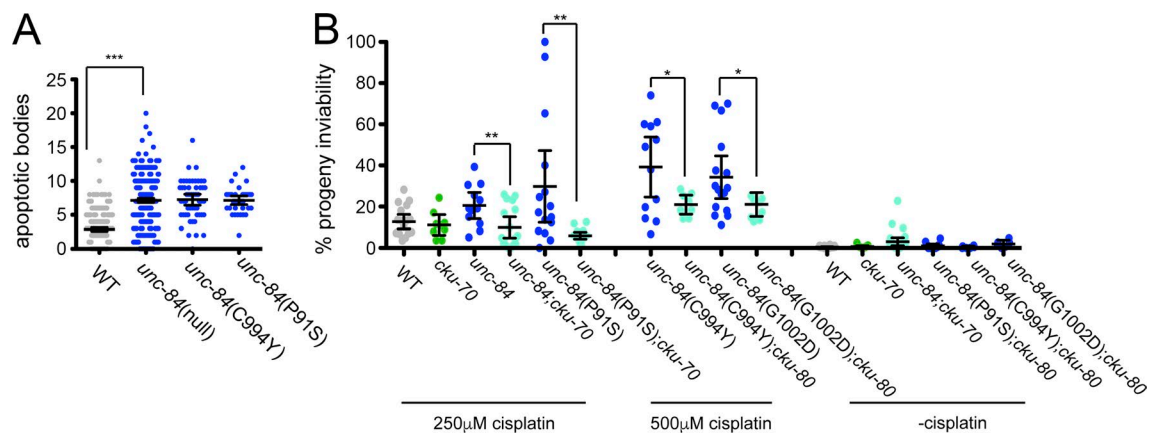


Figure S5. **Embryonic lethality of UNC-84 point mutants after cisplatin is also suppressed by loss of NHEJ.** (A) Number of apoptotic bodies by AO 48 h after L4 in wild-type (WT; N2), *unc-84(n369)*, *unc-84(C994Y)*, and *unc-84(P91S)*. Germline apoptosis is elevated in UNC-84 point mutants. $n > 20$. (B) Percentages of progeny viability in the given genotypes after 250 μ M and 500 μ M cisplatin or without cisplatin. $n > 6$. Loss of *cku-70* suppresses progeny inviability associated with UNC-84 point mutants after cisplatin. *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$.

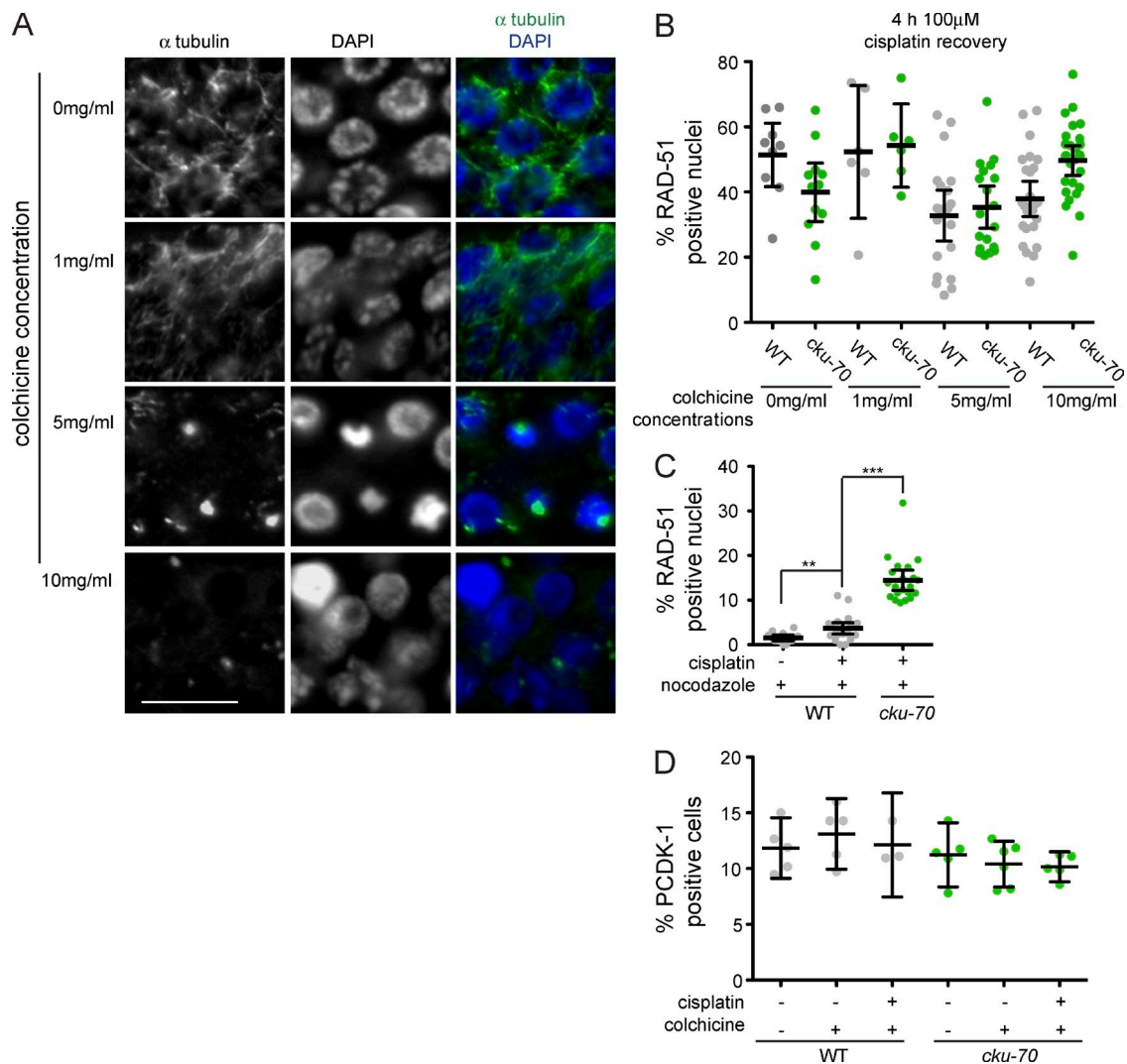


Figure S6. **Microtubule destabilizers affect microtubule network and RAD-51 levels.** (A) Germlines dissected from worms treated with the indicated concentrations of colchicine stained with antibodies against α -tubulin (green) and DAPI (blue). Bar, 10 μ m. (B) Percentages of RAD-51–positive nuclei in wild type (WT) or *cku-70(tm1524)* (after 100 μ M cisplatin) and the given concentrations of colchicine. RAD-51 levels return to wild type after 4 h of cisplatin recovery after microtubule disruption with colchicine. $n > 6$. (C) Percentages of RAD-51–positive nuclei in wild-type and *cku-70(tm1524)* worms treated with cisplatin and nocodazole. Nocodazole also reduces the percentage of RAD-51–positive nuclei, and the reduction can be rescued by the inactivation of NHEJ. $n > 14$. (D) Percentages of PCDK-1–positive nuclei after treatment with colchicine and cisplatin in wild-type and *cku-70(tm1524)* worms. Error bars represent 95% CI. **, $P < 0.01$; ***, $P < 0.0001$. WT, wild type.

References

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