Supplementary Data for

Disabling the Fanconi Anemia Pathway in Stem Cells Leads to Radioresistance and Genomic Instability

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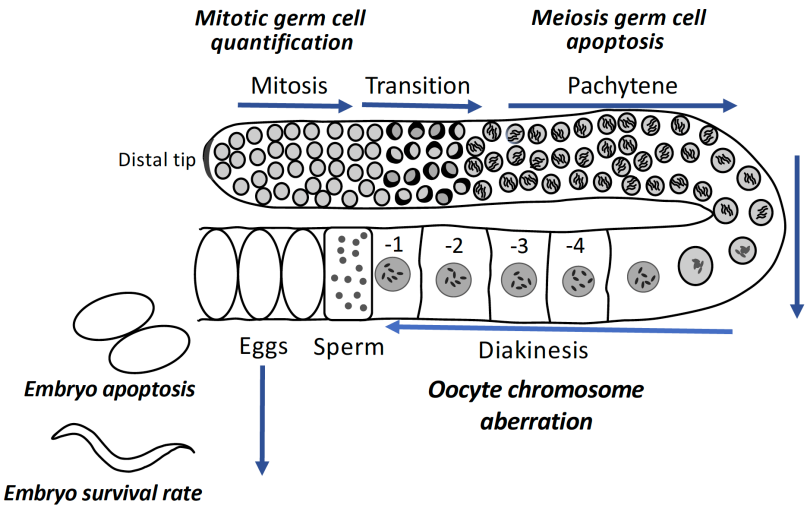
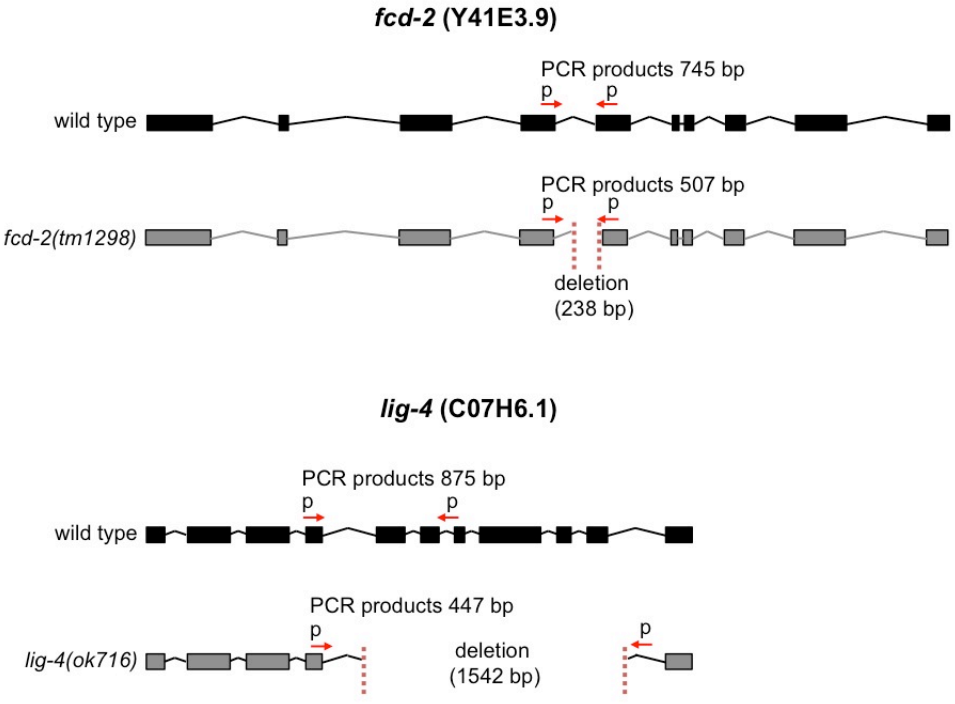


Fig. S1 Schematic drawing of one *C. elegans* gonad arm with germ cell developmental stages. Adult hermaphrodite *C. elegans* contains two U-shaped gonad arms connected by a common uterus. As the blue arrow shows, germ cells proliferate distally (mitotic zone) then enter and progress through meiotic prophase. Proximal diakinesis oocytes are fertilized by sperm as they transit the spermatotheca to initiate embryogenesis. After fertilized eggs are laid, they go through four larval stages (L1–L4) before reaching adulthood. Sensitivity of individual *C. elegans* germ cells to different types of DNA damage varies depending on developmental stage. Assays used to evaluate germ cell DNA damage sensitivity are in bold italic.

A


fcd-2 (Y41E3.9)
PCR products 745 bp
p
p
wild type
PCR products 507 bp
p
p
fcd-2(tm1298)
deletion
(238 bp)
lig-4 (C07H6.1)
PCR products 875 bp
p
p
wild type
PCR products 447 bp
p
lig-4(ok716)
deletion
(1542 bp)
p

B

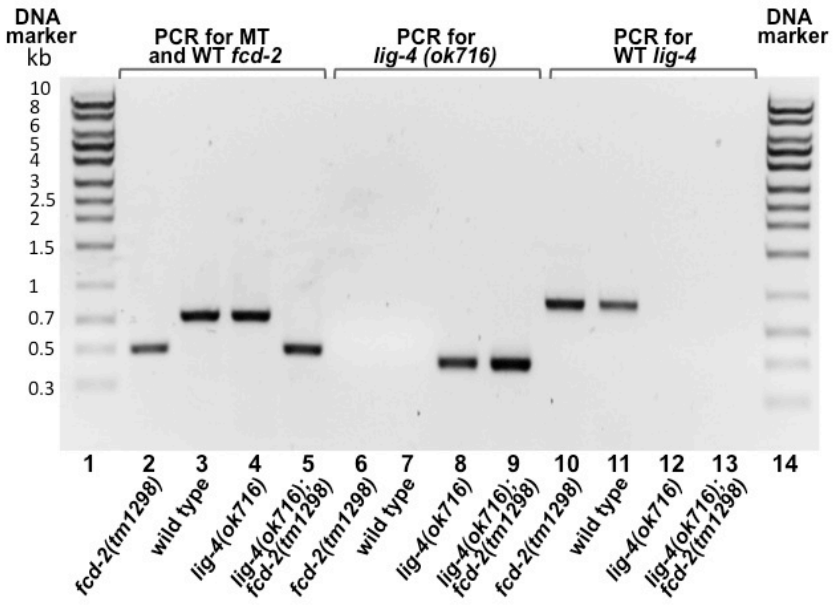

DNA marker kb
PCR for MT and WT *fcd-2*
PCR for *lig-4 (ok716)*
PCR for WT *lig-4*
DNA marker
10
8
6
5
4
3
2.5
2
1.5
1
0.7
0.5
0.3
1 2 3 4 5 6 7 8 9 10 11 12 13 14
fcd-2(tm1298)
wild type
lig-4(ok716)
lig-4(ok716); fcd-2(tm1298)
fcd-2(tm1298)
wild type
lig-4(ok716)
lig-4(ok716); fcd-2(tm1298)
fcd-2(tm1298)
wild type
lig-4(ok716)
lig-4(ok716); fcd-2(tm1298)

Fig. S2 Deletion alleles of *fcd-2(tm1298), lig-4(ok716)* and double mutant *lig-4(ok716);fcd-2(tm1298)*. (**A**) Structural diagrams of *fcd-2* (Y41E3.9) and *lig-4* (C07H6.1). Boxes indicate exons, and lines represent introns. The breakpoint marks the genomic regions of each deletion and red arrows delineate position of primers (p) used for PCR genotyping. (**B**) PCR fragment electrophoresis. In *fcd-2(tm1298)*, a 238 bp fragment from the *fcd-2* gene locus is deleted generating a PCR product of 507 bp (lanes 2, 5) compared with a 745 bp DNA product from wild type and *lig-4(ok716)* worms (lanes 3, 4). With *lig-4* mutant primers the PCR product is 447 bp in *lig-4(ok716)* and *lig-4(ok716);fcd-2(tm1298)* (lanes 8, 9), while there is no PCR product observed in wild-type worms (lanes 6, 7). With *lig-4* wild-type primers, the PCR product is 875 bp in *fcd-2(tm1298)* and wild-type worms (lanes 10, 11), but is absent in *lig-4(ok716)* and *lig-4(ok716);fcd-2(tm1298)* (lanes 12, 13) as the 3' end PCR primer was designed inside the deleted segment of *lig-4*. For primer sequences see Methods.

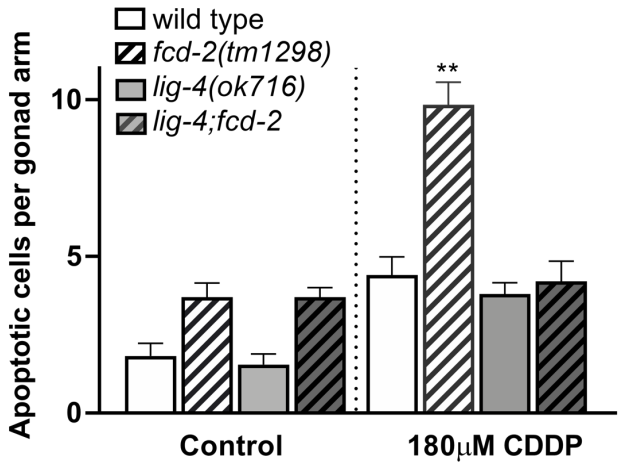


Fig. S3 *lf lig-4* inhibits enhancement of CDDP-induced germ cell apoptosis in a *lf fcd-2* mutant. L4-stage wild type, *fcd-2(tm1298), lig-4(ok176)* and *lig-4;fcd-2* mutants were treated with 180μM CDDP for 16h. Apoptotic germ cells were scored 24h post CDDP. Data (mean±SEM) are from 10-12 animals per group. ***p*≤ *0.01 vs.* CDDP-induced apoptosis in all other groups.

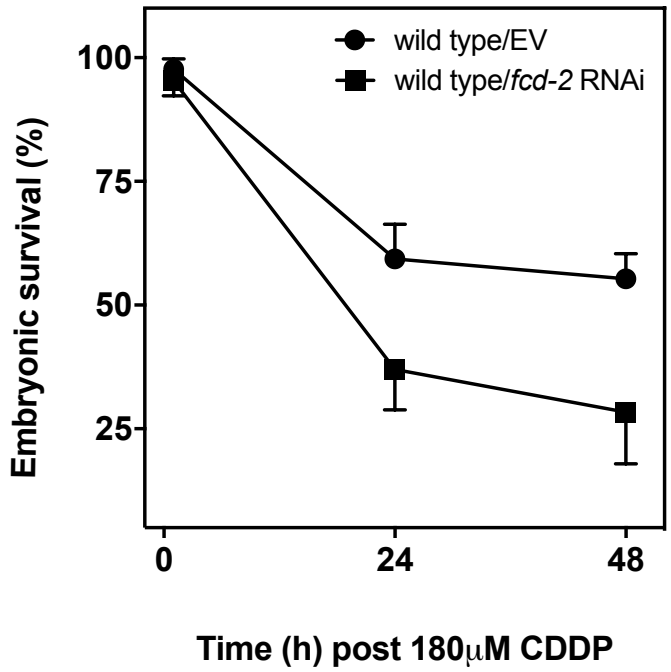


Fig. S4 *fcd-2* knockdown increases CDDP-induced embryonic lethality. To confirm that CDDP-induced embryonic lethality in *fcd-2(tm1298)* results from loss of wild-type *fcd-2*, we knocked down *fcd-2* expression by 30-40 % using feeding RNAi as compared with empty vector (EV)-treated wild-type worms. Progeny survival was scored at 24h and 48h post-CDDP treatment ($p \leq 0.05$ each wild-type/EV *vs.* wild type/*fcd-2* RNAi). Data (mean±SD) are compiled from three independent experiments.

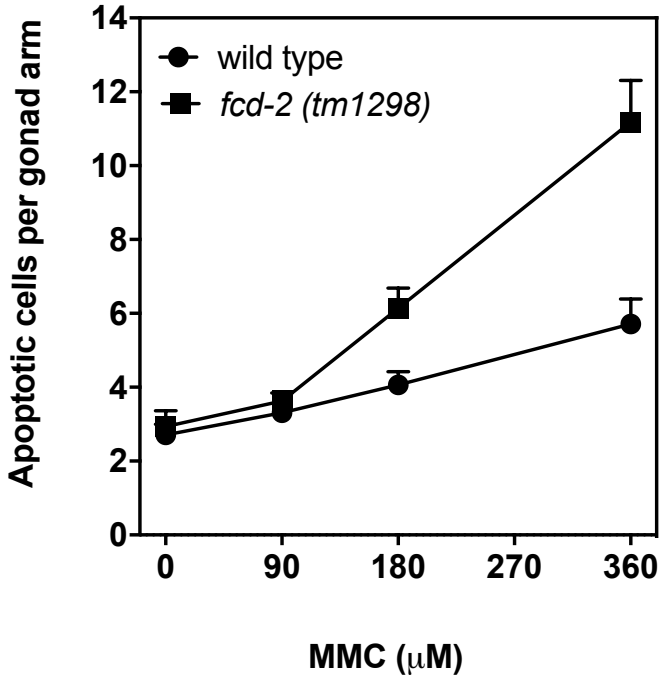


Fig. S5 *fcd-2* mutant worms are hypersensitive to mitomycin C (MMC). L4 stage wild-type *and fcd-2(tm1298)* worms were treated with the indicated doses of MMC for 16h and germline nuclei were quantified after 30h recovery from treatment. Data (mean±SEM) are from ≥ 15 animals per group. $p \leq 0.05$ *fcd-2(tm1298)* vs. wild-type/EV at 180 μM MMC and 360 μM MMC.

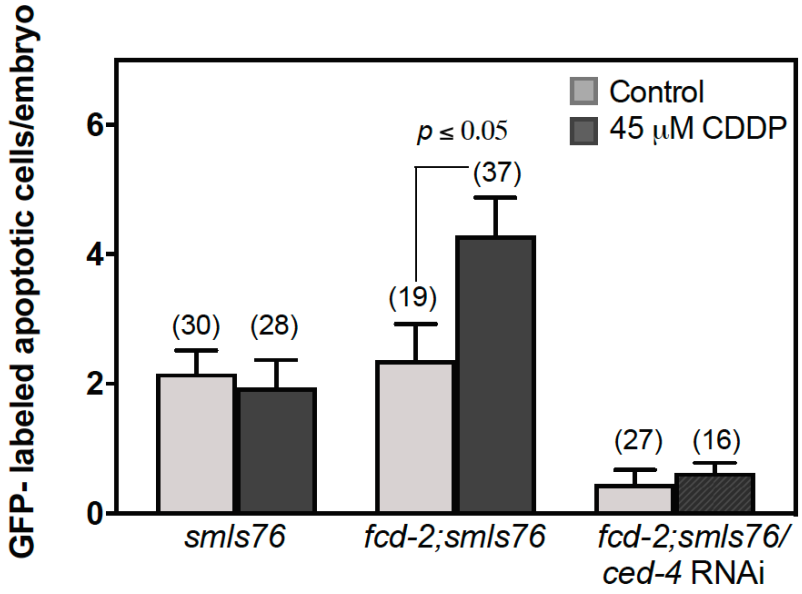


Fig. S6 *ced-4* knockdown blocks embryonic apoptosis in *lf fcd-2*. Late L4-stage worms were treated with 45μM CDDP for 6h, followed by 20h recovery. Thereafter, embryos (number in parentheses) were collected at 2h post-egg laying to detect sAnxV::GFP-expressing apoptotic cells.

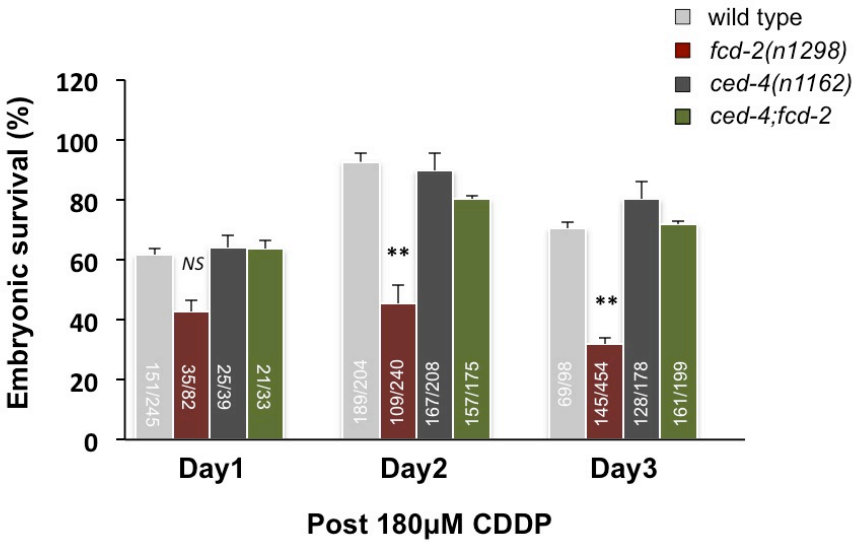


Fig. S7 *lf ced-4* blocks CDDP-enhanced embryonic lethality in an *fcd-2* mutant. Late L4-stage wild type, *fcd-2(tm1298), ced-4(n1162)* and *ced-4;fcd-2* mutants were treated with 180μM CDDP for 16h. Embryonic survival was scored daily for three days post CDDP. Data (mean±SEM) are from 10-15 animals per group and number of hatched eggs over total laid eggs is presented in each column. *p* value was calculated using GraphPad chi square test. *NS* = not significant) and ***p<0.01*, compares survival in *fcd-2(n1298)* with all other groups within the same day.

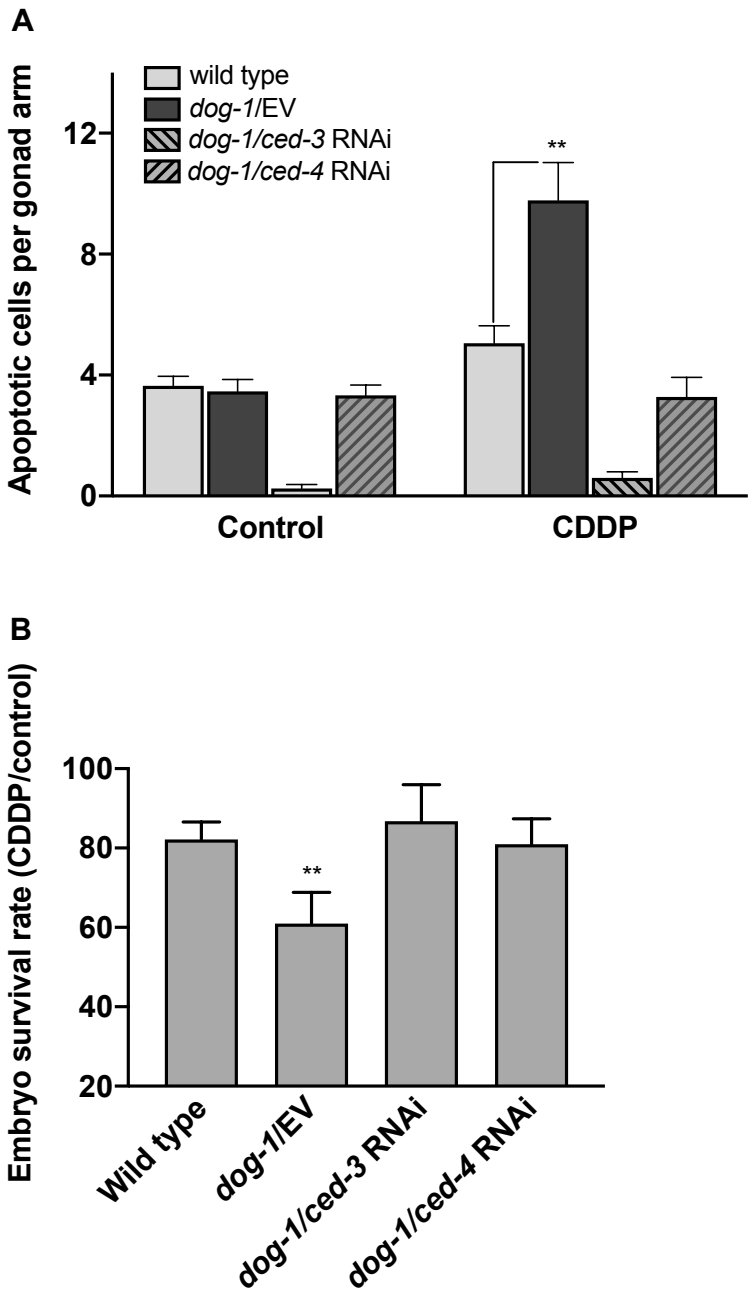


Fig. S8 *lf dog-1*/*Fanc J* increase in CDDP-induced germ cell apoptosis and embryonic lethality are blocked by RNAi *C. elegans* core machinery. Late L4-stage wild-type, *dog-1(gk10),dog-1(gk10)/ced-3* RNAi and *dog-1(gk10)/ced-4* RNAi worms were treated with 180μMCDDP for 16h. (**A**) Apoptotic germ cells were scored at 24h post CDDP. Data (mean ± SEM) are from 12-24 worms per group. (**B**) The embryos were collected 24-48h post CDDP. Embryonic survival rate is presented as the CDDP-treated group compared with its own untreated group in each worm type. Data (mean ± SEM) are from 20-25 animals per group. ** $p<0.01$ in *dog-1(gk10) vs* all other groups.

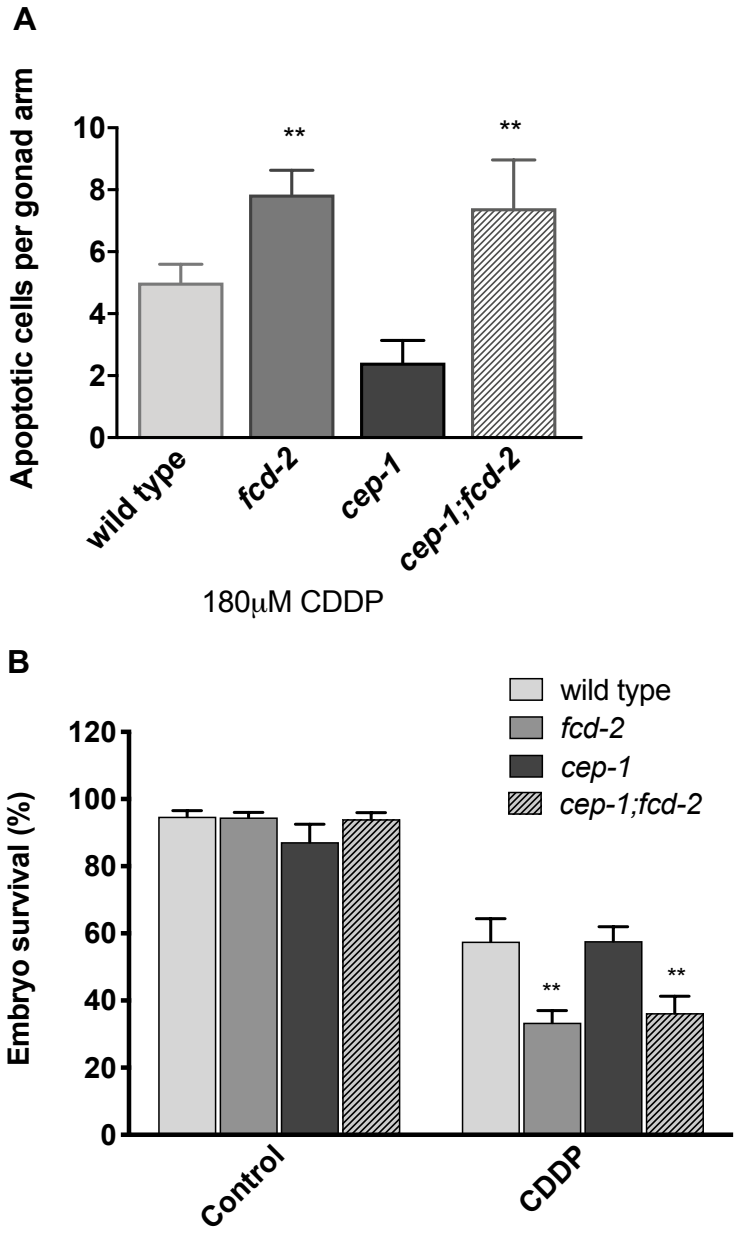


Fig. S9 ICL-induced apoptosis upon *fcd-2* loss-of-function is independent of the *C. elegans* p53 ortholog, CEP-1. (**A)** L4-stage wild-type, *fcd-2(tm1298), cep-1(gk138)* and *cep-1;fcd-2* were treated with 180μM CDDP for 16h. Apoptotic germ cells were scored at 24h post CDDP. Data (mean±SEM) are from 12-15 worms per group. ** *p<0.01* compares number of apoptotic cells in *fcd-2* with wild-type & in *cep-1;fcd-2* with *cep-1*. (**B)** Late L4-stage wild-type, *fcd-2(tm1298), cep-1(gk138)* and *cep-1;fcd-2* mutants were treated with 180μM CDDP for 16h. Embryonic survival was scored over two days post CDDP. Data (mean±SEM) are from 10-15 animals per group. p value was calculated using GraphPad chi square test. ** *p<0.01* compares survival in wild-type with *fcd-2,* and *cep-1* with *cep-1;fcd-2*.

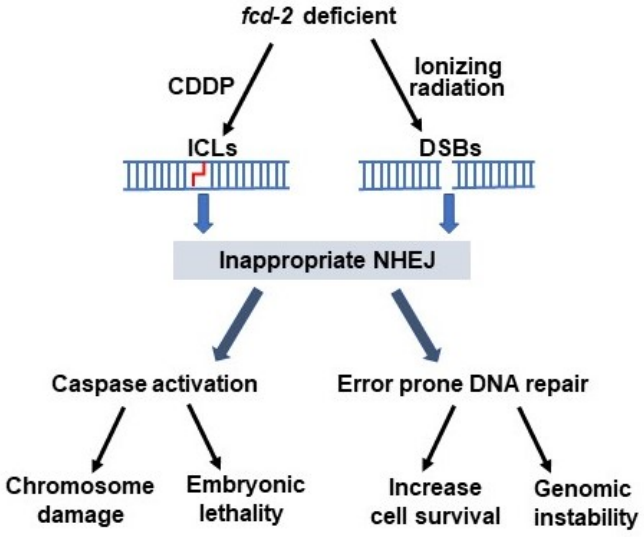


Fig. S10 Inappropriate NHEJ dictates distinct outcomes of intercross link and ionizing radiation DNA damage upon *fcd-2* deficiency. Loss of *fcd-2* function results in inappropriate NHEJ engagement upon DNA damage during meiosis, early embryonic development, and in germline and embryonic stem cells. Type of DNA damage and stage of germline/embryonic development determine outcome. Whereas ICLs do not impact survival of mitotically-active GSCs in FCD-2 deficient *C. elegans*, ICLs activate NHEJ increasing apoptosis in meiosis I and in dividing early stage embryos by an unknown mechanism currently under investigation. Alternately, while ionizing radiation-induced DSBs also engage NHEJ inappropriately, outcomes are different with this form of DNA damage, yielding marked radioresistance in *C. elegans* germline and murine embryonic stem cells. As a multi-generational study in Fanconi-deficient *C. elegans* shows that ionizing radiation but not DNA cross links yields genomic instability, we posit that the predisposition of FA patients toward cancer development reflects enhanced survival of GSCs with pre-neoplastic mutations, while increased anemia may reflect apoptotic death in the hematopoietic compartment.

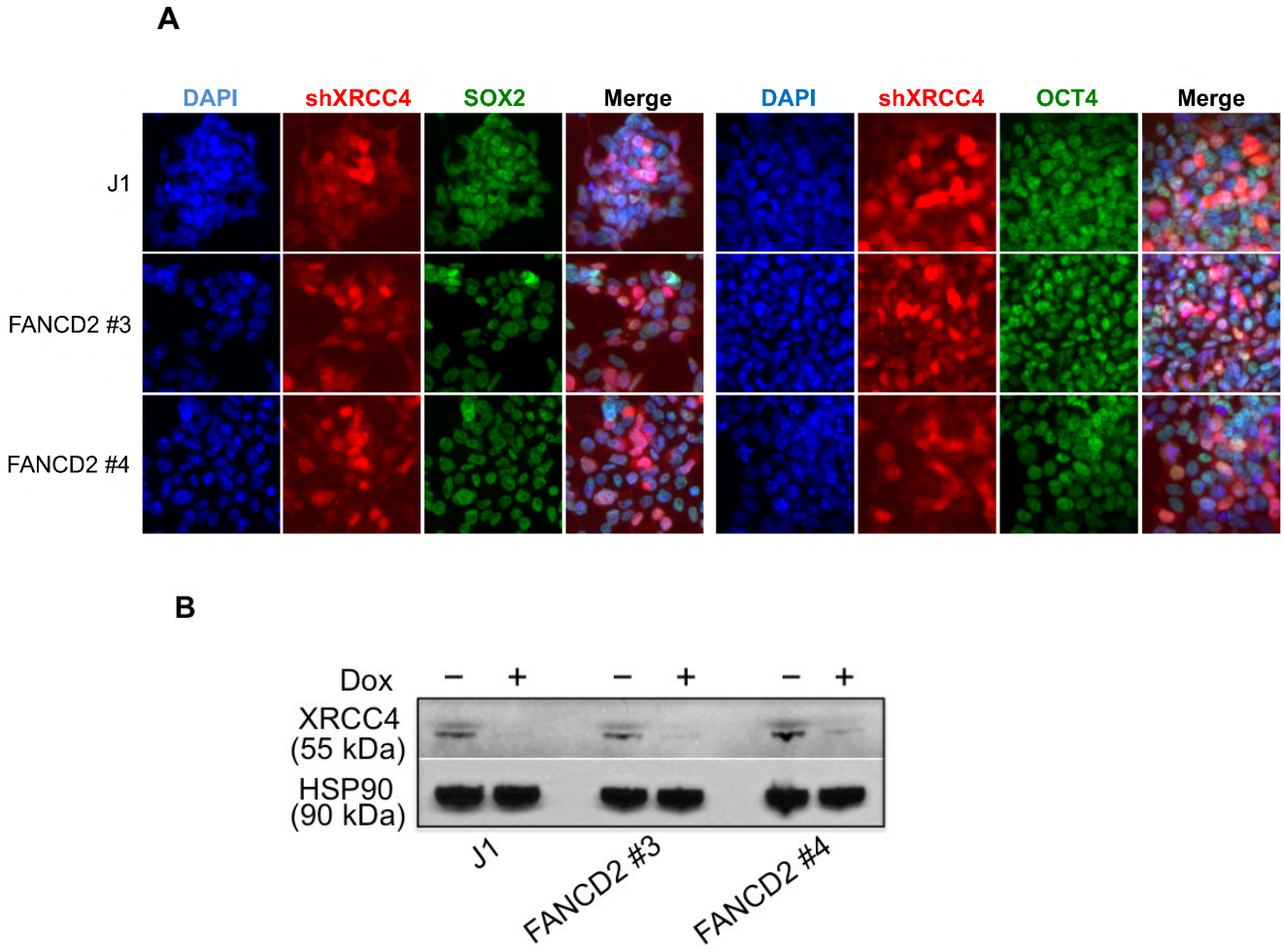


Fig. S11 XRCC4 Knockdown in mouse ES cells. (A) After introducing shRNA expression constructs, selecting with puromycin, and induction by doxycycline, over 80% ESCs express XRCC4 shRNA (red), while ES cell markers, Sox2 (left) and Oct4 (right) remain intact (green). **(B)** Western Blot analysis indicates that upon doxycycline exposure, XRCC4 protein levels decrease to <20% of non-doxycycline exposed XRCC4 shRNA-expressing J1, FANCD#3 and FANCD#4 cells. HSP90, which is unaffected by doxycycline, serves as control.