Supplementary Figure Legends

Supplementary Figure 1 Cell cycle arrest, developmental and physiological apoptosis are functional in *ufd-2* worms. **(a)** Developmental apoptosis in head region of newly hatched L1 larvae of indicated genotypes. Data show means ± s.e.m. of 3 independent experiments. The double and triple asterisks indicate *P* values of ≤ 0.001 and 0.0001 in Student's *t*-test. **(b)** Physiological apoptosis in pachytene region of day 1 adults of indicated genotypes. Data show means ± s.e.m. of 3 independent experiments. The double and triple asterisks indicate *P* values of ≤ 0.001 and 0.0001 in Student's *t*-test. **(c)** Representative DIC images of cell cycle arrest in mitotic region of germlines of indicated genotypes. Worms were imaged 16 hrs after treatment with 60 Gy IR. Filled arrowheads mark enlarged arrested mitotic cells. **(d)** Quantification of cell cycle arrest in mitotic region of germlines. Worms were treated with 60 Gy and analysed 16 hrs later. Data show means ± s.e.m. of 3 independent experiments. The triple asterisks indicates *P* value of 0.0001 in Student's *t*-test, n.s. not significant. For *n*-values see Supplementary Table 2. (e) Autoubiquitylation of UFD-2. Ubiquitylation reactions were

carried out as indicated using UFD-2 (wild-type), UFD-2^{P951A} and UFD-2^{C448Y} as ubiquitin ligases and ubiquitin (wilde-type), ubiquitin$^{only\ K29}$, ubiquitin$^{only\ K48}$ for conjugation.

Supplementary Figure 2 UFD-2 forms foci late after damage induction. **(a)** 150 day 1 adult worms of indicated genotypes were lysed and probed in western blotting with α-UFD-2 and α-TBG-1 antibody. Representative blot, total (n=3). **(b)** Schematic illustration of *C. elegans* germline and illustrative images of a germline stained with α-UFD-2 and DAPI. Two slides from a z-stack (z-slide 1 and z-slide b), one from upper and one from lower part of the germline are shown. Filled arrowheads mark nuclei positive for UFD-2 foci. Scale bar, 10 µm. **(c)** Quantification of UFD-2 foci in pachytene region of wild-type germlines. Worms were treated with 0 and 60 Gy IR and isolated 5 and 24 hrs later. Data show means of ± s.e.m. of ≥ three experiments. For *n*-values see Supplementary Table 2.

Supplementary Figure 3 UFD-2 foci formation is IR dependent. **(a)** Quantification of UFD-2 foci in pachytene region of wild-type germlines. Worms were treated with 0, 60 Gy of IR or 600 J/m^2 of UV and analyzed 24 hrs later. Data show means ± s.e.m. of 3 experiments. **(b)** L4 stage larvae were irradiated with 0, 30 or 60 Gy of IR and scored for embryonic survival (number of hatched larvae normalized to results after mock-treatment). Data show means ± s.e.m. of ≥ five experiments. The single, double and triple asterisks indicate *P* values of ≤ 0.05, 0.001, 0.0001 in Student's *t*-test. For *n*-values see Supplementary Table 2. **(c)** Representative images of worm germlines of indicated genotypes irradiated with 60 Gy IR and stained with α-ATX-3 antibody and DAPI 24 hrs later. Scale bar, 5 µm. Representative images of 3 independent experiments. **(d)** Day 1 adult worms of indicated genotypes were lysed and probed in western blotting with α-ATX-3 and α-TBG-1 antibody. Representative blot, total (n=3). **(e)** Quantification of number of UFD-2 foci that co-localise with ubiquitin in wild-type germlines. Worms were treated with 60 Gy of IR and analyzed 24 hrs later. Data show means ± s.e.m. of 3 experiments. **(f)** Sequence alignment shows UFD-2 from C. elegans

and other species, the conserved residue C448 is highlighted. **(g)** *in vivo* co-immunoprecipitation of CDC-48 with UFD-2 from indicated worm lysates. **(h)** *in vitro* co-immunoprecipitation of CDC-48 with UFD-2 with purified recombinantly expressed protein. **(i)** Autoubiquitylation of UFD-2. Ubiquitylation reactions were carried out as indicated using UFD-2 (wild-type), UFD-2^{P951A} and UFD-2^{C448Y} as ubiquitin ligases. **(j)** Quantification of UFD-2 foci in pachytene region of indicated germlines. Worms were treated with 0 and 60 Gy IR and isolated 24 hrs later. Data show means of ± s.e.m. of three experiments. For *n*-values see Supplementary Table 2. **(k)** Quantification of ubiquitin foci in pachytene region of indicated germlines. Worms were treated with 60 Gy IR and isolated 24 hrs later. Data show means of ± s.e.m. of three experiments. For *n*-values see Supplementary Table 2.

Supplementary Figure 4 Apoptosis induction in *ufd-2* is functional. **(a)** Representative images of worm germlines of indicated genotypes irradiated with 60 Gy IR and stained with α-UFD-2 antibody and DAPI 24 hrs later. Empty and filled arrowhead indicated nuclei positive or negative for UFD-2 foci, respectively. Scale bar, 5 µm. **(b)** Worms were treated with 0 and 60 Gy of IR and 150 worms of indicated genotypes were lysed and probed in western blotting with α-CEP and α-TBG-1 antibody. Representative blot, total (n=3) and **(c)** quantification of three independent blots. **(d)** Relative expression levels of *egl*-1 target gene in wild-type and *ufd-2(tm1380)* worms at indicated time points after treatment with 0 or 60 Gy IR. mRNA levels were normalized to 0 Gy samples. Data show means ± s.e.m. of 3 independent experiments. **(e)** Quantification of germ cells positive for RAD-51 staining. Wild-type and *atx-3(gk193)* worms were treated with 0 or 20 Gy of IR and isolated 24 hrs after treatment and immunostained with α-RAD-51 and DAPI. The last 50 nuclei of pachytene germ cells prior entering diakinesis were evaluated. Data show means ± s.e.m. of 3 independent experiments. The single asterisk indicates *P* value of ≤ 0.01 in Student's *t*-test. For *n*-values see Supplementary Table 2.

Supplementary Figure 5 UFD-2 foci are dependent on apoptosis and DNA damage signalling. **(a)** Representative images of worm germlines of indicated genotypes irradiated with 60 Gy IR and stained with α-UFD-2 antibody and DAPI 24 hrs later. Empty and filled arrowhead indicated nuclei positive or negative for UFD-2 foci, respectively. Scale bar, 5 µm. **(b)** Worms were treated with 0 and 60 Gy of IR and 150 worms of indicated genotypes were lysed and probed in western blotting with α-UFD-2 and α-TBG-1 antibody. Representative blot, total (n=3). **(c)** Quantification of germ cells positive for RAD-51 staining. Worms were treated with 0 or 20 Gy of IR and isolated 24 hrs after treatment and immunostained with α-RAD-51 and DAPI. The last 50 nuclei of pachytene germ cells prior entering diakinesis were evaluated. Data show means ± s.e.m. of 3 independent experiments. For *n*-values see Supplementary Table 2.

Supplementary Figure 6 (a) L4 stage larvae were irradiated with 0 or 60 Gy of IR and scored for embryonic survival (number of hatched larvae normalized to results after mock-treatment). Data show means ± s.e.m. of three experiments. The triple asterisk indicates *P* value of ≤ 0.0001 in Student's *t*-test, *n.s.* not significant. For *n*-values see Supplementary Table 2.

Supplementary Figure 7 Full scanned images of immunoblots presented in figures. **(a)** Presented in Figure 1e. **(b)** Presented in Supplementary Figure 1e. **(c)** Presented in Supplementary Figure 2a. **(d)** Presented in Supplementary Figure 3d. **(e)** Presented in Supplementary Figure 3g. **(f)** Presented in Supplementary Figure 3h. **(g)** Presented in Supplementary Figure 3i. **(h)** Presented in Supplementary Figure 4b.

Supplementary Table 1 *n*-values for Figures 1-6.

Supplementary Table 2 *n*-values for Supplementary Figures 1-6.