

717 **Supplementary Figure Legends**

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

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

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

742 **Supplementary Figure 3** UFD-2 foci formation is IR dependent. **(a)** Quantification of UFD-
743 2 foci in pachytene region of wild-type germlines. Worms were treated with 0, 60 Gy of IR or
744 600 J/m² of UV and analyzed 24 hrs later. Data show means \pm s.e.m. of 3 experiments. **(b)** L4
745 stage larvae were irradiated with 0, 30 or 60 Gy of IR and scored for embryonic survival
746 (number of hatched larvae normalized to results after mock-treatment). Data show means \pm
747 s.e.m. of \geq five experiments. The single, double and triple asterisks indicate *P* values of \leq
748 0.05, 0.001, 0.0001 in Student's *t*-test. For *n*-values see Supplementary Table 2. **(c)**
749 Representative images of worm germlines of indicated genotypes irradiated with 60 Gy IR
750 and stained with α -ATX-3 antibody and DAPI 24 hrs later. Scale bar, 5 μ m. Representative
751 images of 3 independent experiments. **(d)** Day 1 adult worms of indicated genotypes were
752 lysed and probed in western blotting with α -ATX-3 and α -TBG-1 antibody. Representative
753 blot, total (n=3). **(e)** Quantification of number of UFD-2 foci that co-localise with ubiquitin in
754 wild-type germlines. Worms were treated with 60 Gy of IR and analyzed 24 hrs later. Data
755 show means \pm s.e.m. of 3 experiments. **(f)** Sequence alignment shows UFD-2 from *C. elegans*

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

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

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

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

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

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

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