ALG-2/AGO-dependent *mir-35* family regulates DNA damage-induced apoptosis through MPK-1/ERK MAPK signaling downstream of the core apoptotic machinery in *C. elegans*

Markus Alexander Doll[1,2], Najmeh Soltanmohammadi[1,2] and Björn Schumacher[1,2]

[1]Institute for Genome Stability in Ageing and Disease, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany

[2]Cologne Excellence Cluster for Cellular Stress Responses in Ageing-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany

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Corresponding author: Björn Schumacher, Cologne Excellence Cluster for Cellular Stress Responses in Ageing-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany, Tel. +49 221 478 84202, bjoern.schumacher@uni-koeln.de

SUPPLEMENTAL FIGURE LEGENDS

Figure S1 | *alg-2(ok304)* mutants display numerous apoptotic corpses accumulating in the proximal germline 4 h post-IR.

(A) Acridine orange staining revealed numerous apoptotic corpses within the proximal germline half of IR-treated *alg-2* mutant animals. Representative fluorescent and DIC images of IR- and untreated WT and *alg-2(ok304)* mutant germlines taken at 4 h post-IR treatment (90 Gy). Scale bars correspond to 20 µm. (B) DAPI-staining of germ cell nuclei accumulating in the proximal germline half of IR-treated *alg-2(ok304)* mutant animals. Germlines were dissected and stained with DAPI 4 h post-IR (90 Gy). Representative images were generated from Z-stacks taken via confocal microscopy. Scale bars correspond to 40 µm.

Figure S2 | Cytoplasmic ALG-2::HA expression in *alg-2;palg-2::ALG-2::HA* germlines.

Germlines of WT, *alg-2(ok304)* and *alg-2;palg-2::ALG-2::HA* animals were dissected and stained with either of the two indicated anti-HA antibodies. Representative images were generated from Z-stacks taken via confocal microscopy. Scale bars correspond to 40 µm in full germline images (left) and 20 µm in close-up pictures (right).

Figure S3 | *alg-2* mutant animals exhibit mild HR-directed repair defect and a subtle delay in mitotic cell cycle arrest upon IR exposure.

(A) Early *alg-2(ok304)* embryos displayed slightly reduced survival after IR-treatment compared to the survival of the WT embryos, suggesting less efficient HR-directed repair in this mutant. Graph summarizes results from nine independent experiments, each done in triplicate. (*** = P<0.001, **** = P<0.0001; two-way ANOVA, error bars show SD) (B) *alg-2(ok304)* mutant germlines exhibit a slight increase in the number of persistent RAD-51 foci 16 hours post-IR (30 Gy) compared to that of WT, indicative of higher levels of unresolved DNA DSBs. Sample sizes: WT (untreated): N=16, WT (IR-treated): N=24, *alg-2(ok304)* (untreated): N=17, *alg-2(ok304)* (IR-treated): N=28; medians with 95% CI are shown (** = P<0.01; Mann-Whitney non-parametric test). (C) Elevated numbers of mitotic cells in the distal germline suggest that *alg-2(ok304)* mutant animals have a slight delay of checkpoint activation after exposure to 30 Gy of IR that is lost at higher doses. (Representative graph from one out of four independent experiments with 5 scored germlines per genotype and condition, * = P<0.05; two-way ANOVA)

Figure S4 | EGL::V5 can trigger apoptosis in consequence to DNA damage.

egl-1;(syb1272 [egl-1::V5]) and *alg-2(ok304);egl-1(syb1272 [egl-1::V5])* animals exhibit a reduced apoptotic response upon genotoxic stress. Apoptosis was scored 4 hours post-IR treatment (90 Gy). (Medians with 95% CI are shown, 5-10 germlines per genotype and condition were scored. * = P<0.05, **** = P<0.0001; Mann-Whitney non-parametric test).

Figure S5 | SIR-2.1 partially contributes to DNA damage-induced apoptosis in *alg-2* mutant worms.

(A) Dissected germlines from *alg-2(ok304)* mutant animals display severe loss of the nuclear SIR-2.1 signal in late-pachytene germ cells (area surrounded by dashed line) that depended on functional CED-3 activity. Germlines were dissected at 2.5 h post-IR or mock treatment. Representative images were generated from Z-stacks taken via confocal microscopy. Scale bars correspond to 20 µm. (B) *sir-2.1(ok434)* suppressed WT cell death levels in response to DNA damage but only partially rescued the *alg-2(ok304)* apoptosis phenotype. Apoptosis was scored 4 hours post-IR treatment (90 Gy). (Representative graphs from one out of three independent experiments, medians with 95% CI are shown, a minimum of 15 germlines per genotype and condition were scored. **** = P<0.0001; Mann-Whitney non-parametric test). (C) RNAi-mediated *sir-2.1* knockdown confirms the specificity of the SIR-2.1 antibody used in A. Germlines were dissected at 2.5 h post-IR or mock treatment. Representative images were generated from Z-stacks taken via confocal microscopy. Scale bars correspond to 20 µm.

Figure S6 | MPK-1 is hyperphosphorylated in *alg-2* mutant germline loops upon IR exposure.

Representative images of WT and *alg-2(ok304)* mutant germlines dissected 2.5 h after IR (90 Gy) or mock-treatment and stained with an antibody recognizing phosphorylated MPK-1. The mid-pachytene phospho-MPK-1 signal is highlighted by yellow dashed lines. The loop region of each germline

is highlighted by white dashed lines. Pictures were generated from Z-stacks taken via confocal microscopy. Images used in Fig. 4A were included. Scale bars correspond to 40 µm.

Figure S7 | Reduced ERK MAPK signaling partially rescues elevated apoptosis in *alg-2(ok304)* mutant animals after exposure to 90 Gy of γ-irradiation.

Three separate experiments illustrate a significant yet variable rescue of the *alg-2(ok304)* mutant apoptosis phenotype due to reduced ERK activity upon 90 Gy of γ-irradiation; medians with 95% CI are shown, a minimum of 15 germlines per genotype and condition were scored. (n.s. = P>0.05, * = P<0.05, **** = P<0.0001; Mann-Whitney non-parametric test)

Figure S8 | *cep-1(gk138)* deletion abolishes MPK-1 phosphorylation in IR-treated *alg-2(ok304)* mutant germline loops.

(A) IR-induced MPK-1 phosphorylation was completely eliminated in the gonad loops of the *alg-2(ok304);cep-1(gk138)* double mutant animals. Germlines were dissected 2.5 hours post-IR treatment with 90 Gy. Representative images were generated from Z-stacks taken via confocal microscopy, germline loop regions are highlighted by dashed lines, scale bars correspond to 20 µm. (B) Quantification of WT, *alg-2(ok304)* and *alg-2(ok304);cep-1(gk138)* mutant germlines displaying induced MPK-1-phosphorylation in the late-pachytene zone or the entire gonad loop 2.5 h

after IR exposure (90 Gy). Phospho-MPK-1 signal was assessed at 10x and 40x magnification with a fluorescent microscope. Sample sizes are indicated.

Figure S9 | *ced-9(n1950)* gain of function abolishes MPK-1 phosphorylation in IR-treated *alg-2(ok304)* mutant germline loops.

(A) IR-induced MPK-1 phosphorylation was completely eliminated in gonad loops of the *alg-2(ok304);ced-9(n1950)* double mutant animals. Germlines were dissected 2.5 hours post-IR treatment (90 Gy). Representative images were generated from Z-stacks taken via confocal microscopy, germline loop regions are highlighted by dashed lines, scale bars correspond to 20 µm. (B) Quantification of WT, *alg-2(ok304)* and *alg-2(ok304);ced-9(n1950)* mutant germlines displaying induced MPK-1-phosphorylation in the late-pachytene zone or the entire gonad loop 2.5 h after IR exposure (90 Gy). Phospho-MPK-1 signal was assessed at 10x and 40x magnification with a fluorescent microscope. Sample sizes are indicated. (C) *ced-9(n1950)* fully abrogated IR-induced germ cell apoptosis in WT and *alg-2(ok304)* mutant animals. Apoptosis was scored 4 h post-IR treatment (90 Gy). (Representative graph from one out of three independent experiments, medians with 95% CI are shown, a minimum of 15 germlines per genotype and condition were scored. **** = $P<0.0001$; Mann-Whitney non-parametric test).

Figure S10 | Weak *ced-3* mutant alleles block MPK-1 phosphorylation in the gonad loop of *alg-2(ok304)* mutant animals.

(A) IR-induced MPK-1 phosphorylation was completely abolished in gonad loops of the *alg-2(ok304);ced-3(op149)* and *alg-2(ok304);ced-3(n2438)* double mutant animals. Germlines were dissected 2.5 h post-IR treatment (90 Gy). Representative images were generated from Z-stacks taken via confocal microscopy, germline loop regions are highlighted by dashed lines, scale bars correspond to 20 µm. (B) Quantification of WT, *alg-2(ok304)*, *alg-2(ok304);ced-3(op149)* and *alg-2(ok304);ced-3(n2438)* mutant germlines displaying induced MPK-1-phosphorylation in the late-pachytene zone or the entire gonad loop 2.5 h after IR exposure (90 Gy). Phospho-MPK-1 signal was assessed at 10x and 40x magnification with a fluorescent microscope. Sample sizes are indicated. (C) Weak loss-of-function alleles *ced-3(op149)* and *ced-3(n2438)* nearly eliminated apoptosis in the WT and *alg-2(ok304)* mutant germlines. Apoptosis was scored 4 h post-IR treatment (90 Gy). (Representative graph from one out of three independent experiments, medians with 95% CI are shown, a minimum of 15 germlines per genotype and condition were scored. **** = P<0.0001; Mann-Whitney non-parametric test).

Figure S11 | MPK-1 phosphorylation is suppressed in CED-1::GFP-positive late-pachytene germ cells undergoing apoptosis.

(A) Only a subfraction of CED-1::GFP-labeled apoptotic germ cells retained phosphorylated MPK-1. In the majority of cells, engulfment abrogated MPK-1 activity. Germlines were dissected 2.5 h post-IR treatment (90 Gy). Representative images were taken via confocal microscopy, scale bars correspond to 20 µm.

Figure S12 | MPK-1 phosphorylation is abrogated in late apoptotic corpses in the *alg-2(ok304)* mutant germline.

Persistent late apoptotic corpses in the proximal half of *alg-2(ok304)* mutant germlines no longer exhibited phosphorylated MPK-1. Germlines were isolated 4 hours post IR-treatment (90 Gy). Representative images were taken via confocal microscopy, late corpses are highlighted by dashed boxes, scale bars correspond to 20 µm.

Supplementary table 1: pdf-format: miRNA regulation wild type IR-treated vs wild type untreated

Supplementary table 2: pdf-format: miRNA regulation *alg-2(ok304)* untreated vs wild type untreated

Supplementary table 3: pdf-format: miRNA regulation *alg-2(ok304)* IR-treated vs *alg-2(ok304)* untreated

Supplementary table 4: pdf-format: miRNA regulation *alg-2(ok304)* IR-treated vs wild type IR-treated