Supplementary Information 5

Abbreviations for each organism in Figure 1A

cel: Nematode worm (*Caenorhabditis elegans*), cbr: Nematode worm (*Caenorhabditis briggsae*), ame: Honey bee (*Apis mellifera*), mmu: House mouse *(Mus musculus*), dre: Zebrafish *(Danio rerio)*, bta: Cattle *(Bos taurus),* hsa: Human (*Homo sapiens*), xtr: Xenopus *(Xenopus tropicalis),* gga: Chicken *(Gallus gallus)*, dme: Fruit fly (*Drosophila melanogaster*), bmo: Silkworm *(Bombyx mori),* cin: Sea squirt *(Ciona intestinalis)*

Additional Information on Materials and Methods

C. elegans strains and growth.

C. elegans were grown under standard conditions. *mir-34* loss-of-function mutant alleles *gk437* and *n4276* were obtained from CGC and backcrossed nine times and three times to wild-type N2, respectively. The *cep-1* alleles were also obtained from CGC, and *gk138* allele was crossed with *mir-34*(*gk437*) for making double mutants.

C. elegans mir-34 RT-PCR and Northern blots.

Total RNA was isolated at three hours after treated with 200 Gy of radiation at the first S-phase radioresistance peak (Weidhaas et al., 2006). TaqMan small RNA assay (Applied Biosystems) was used for RT-PCR. We repeated the experiments three times independently to examine miR-34 level in wild-type and in *cep-1*(*gk138*) post-irradiation (Fig.2). For northern blot, a 22 nucleotide oligo-DNA complementary to mature *mir-34* was ordered from IDT (Integrated DNA Technologies) and labeled using the StarFire labeling kit (IDT).

C. elegans Radiation and Cell Death Assays.

For the vulva cell death model, staged larval animals were treated with radiation at the first S-phase radioresistance peak. The experiments shown in Fig.3B and 3D were repeated three times. For apoptosis assays, animals were treated in the late L4 with 60 and 120 Gy of radiation as described in (Gartner et al., 2000), and the number of apoptotic bodies were scored in three independent experiments.

Plasmid construction and generation of transgenic animals.

Approximately 6.6 kb of DNA in the *mir-34* upstream region (up to next gene, Y41G9A.3), was amplified by PCR and sub-cloned into a *gfp* vector, pPD95.70 to generate pmir-34gfp#60. The transgenic animals, (*zaIs4* carrying the *mir-34* promoter::*gfp* line Is03-24), were made by microinjection of the plasmid pmir-34gfp#60 DNA together with the injection marker *rol-6* followed by integration of the extra-chromosomal array chromosome.

Quantitative Real-time PCR for miR-34a expression in breast cell lines.

Various breast cell lines were grown as described above and total RNA was isolated using mirVana^{TM} miRNA Isolation Kit (Ambion) following the protocol. Taqman microRNA Assay (ABI, per standard protocol) was used to detect and quantify mature mature hsa-miR-34a. Normalization was performed with the small nuclear RNA U6 (RNU6B; ABI). Comparative real-time PCR was performed in triplicate, including no template controls. Relative expression of miR-34a in breast cancer lines to the normal breast epithelial lines was calculated using the $2^{-\Delta\Delta CT}$ method and (CT values from three separate experiments were) analyzed by ANOVA and Tukey's multiple comparison test. The results are depicted as either the mean log of intensities or as a relative value of the mean for control (Fig.4A and Supplementary Information 4A).

Tissue culture and assays in human cells.

Breast cells were grown under standard conditions using MEBM supplemented with BPE, hEGF, insulin, hydrocortisone and GA-1000 for HMEC and MCF10A lines. MDA-MB-231 cells were grown in Leibkovitz medium supplemented with 10% FBS and 1% penicillin-streptomycin. Assays for apoptosis in cell lines include Caspase-3/CPP32 colorimetric assay and western blots to detect PARP cleavage. 3×10^5 cells plated in 6cm culture dishes were irradiated (5, 10 an 15Gy) using the X-RAD 320 x-ray machine. Cell lysates were collected 8 and 24h post irradiation. CPP32 assays were performed according to the kit instructions (Biovision) and the fold-increase in CPP32 activity post irradiation was compared to the un-irradiated controls. Western blots were performed to detect the levels of cleaved PARP using the rabbit polyclonal PARP antibody (1:1000, Cell Signaling Technology Inc). Clonogenic assays were performed using standard conditions, as previously described in (Weidhaas et al., 2007). Briefly, cells were transfected with the miRNA, antimir or control mir using

X-tremeGENE. Twenty-four hours later cells were treated with radiation, and then plated and colonies were scored after 2 weeks. As for statistical analysis in caspase 3 colorimetric cell death assay in Jurkat cells post irradiation (Fig.4C), the OD values obtained from three independent experiments were analyzed by one-way ANOVA and Newman-Keuls multiple comparison test.