## SUPPLEMENTARY MATERIAL



**Supplementary Figure 1. Confirmation of combination with DDR-related factors knockdown and Diap1 overexpression.** Flies carrying  $Myo^{ts}>GFP$ ,  $Myo^{ts}>GFP+Rad50i$ ,  $Myo^{ts}>GFP+Rad50i+Diap1$ ,  $Myo^{ts}>GFP+ATRi$ ,  $Myo^{ts}>GFP+ATRi+Diap1$ ,  $Myo^{ts}>GFP+Chk2i$ ,  $Myo^{ts}>GFP+Chk2i+Diap1$ , or  $Myo^{ts}>GFP+Diap1$  genotypes were fed media with 25 µg/ml Bleomycin at 29 °C for 2 days. The guts of flies were dissected and labeled with anti-GFP (green) and anti- $\gamma$ H2AvD (red) antibodies and DAPI (blue). A'-A''', B'-B''', C'-C''', D'-D''', E'-E''', F'-F''', G'-G''', H'-H''', I'-I''', and J'-J''' were gray scale image of in A-J, respectively. Original magnification is 400×.



Supplementary Figure 2. Effect of Diap1 overexpression on EC-specific knockdown of DDR-related factors-induced

**of EC death.** Flies carrying *Myo<sup>ts</sup>>GFP*, *Myo<sup>ts</sup>>GFP+Rad50i*, *Myo<sup>ts</sup>>GFP+Rad50i+Diap1*, *Myo<sup>ts</sup>>GFP+ATRi*, *Myo<sup>ts</sup>>GFP+ATRi*, *Myo<sup>ts</sup>>GFP+Chk2i*, *Myo<sup>ts</sup>>GFP+Chk2i+Diap1*, or *Myo<sup>ts</sup>>GFP+Diap1* genotypes were cultured at 29 °C for 4 days. The guts of flies were dissected and labeled with anti-GFP (green) and anti-Cleaved Caspase-3 (red) antibodies and DAPI (blue). A'-A''', B'-B''', C'-C''', D'-D''', E'-E''', F'-F''', G'-G''', and H'-H''' were enlarged image of white square in A-H, respectively. A''-A''', B''-B'''', C''-C'''', D''-D'''', E''-E'''', F''-F'''', G''-G'''', and H''-H''' were gray scale image of in A'-H', respectively. Original magnification is 400×.



Supplementary Figure 3. Effect of Diap1 overexpression on EC-specific knockdown of DDR-related factors-induced DNA damage accumulation in ISCs. Flies carrying  $Myo^{ts}>GFP$ ,  $Myo^{ts}>GFP+Rad50i$ ,  $Myo^{ts}>GFP+Rad50i+Diap1$ ,  $Myo^{ts}>GFP+ATRi$ ,  $Myo^{ts}>GFP+ATRi+Diap1$ ,  $Myo^{ts}>GFP+Chk2i$ ,  $Myo^{ts}>GFP+Chk2i+Diap1$ , or  $Myo^{ts}>GFP+Diap1$  genotypes were cultured at 29 °C for 4 days. The guts of flies were dissected and labeled with anti-GFP (green), anti-Delta (white), and anti- $\gamma$ H2AvD (red) antibodies and DAPI (blue). A'-A'''', B'-B'''', C'-C'''', D'-D'''', E'-F'''', G'-G'''', and H'-H''' were gray scale image of in A-H, respectively. Yellow arrow heads indicate Delta<sup>+</sup> cell. Original magnification is 400×.

## SUPPLEMENTARY MATERIALS AND METHODS

## Flies

UAS-Diap1 were kindly provided by the Bloomington Stock Center. Fly genotypes Myo1A-GAL4/+;UAS-GFP,tub-Gal80<sup>ts</sup>/+ Myo1A-GAL4/UAS-Rad50-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/+ Myo1A-GAL4/UAS-Rad50-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/UAS-Diap1 Myo1A-GAL4/UAS-ATR-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/+ Myo1A-GAL4/UAS-Chk2-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/+ Myo1A-GAL4/UAS-Chk2-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/+ Myo1A-GAL4/UAS-Chk2-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/+ Myo1A-GAL4/UAS-Chk2-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/+ Myo1A-GAL4/UAS-Chk2-RNAi;UAS-GFP,tub-Gal80<sup>ts</sup>/+

## **Bleomycin feeding assay**

Three-day-old flies were fed 25  $\mu$ g/ml Bleomycin (Sigma-Aldrich) in normal media for 2 days at 29 °C.