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Supplementary Information for

Trade-off between somatic and germline repair in a vertebrate supports the "expensive germline" hypothesis

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This PDF file includes:

Supplementary Information Text

Methods

Procedure for fixing and sectioning paraffin-embedded adult zebrafish. Fish samples were prepared by removing the head and the tail, followed by slitting open the body cavity to allow uniform and complete fixation of the organs. Samples were then fixed in Bouin's solution on a rotor in 4°C overnight. The next morning, Bouin's solution was removed and samples were washed with 70% ethanol on a rotor for 10 minutes in room temperature for three times. After washing, samples were briefly rinsed with fresh 70% ethanol before being dehydrated using the following steps:
70% ethanol, 10 minutes, room temperature;
70% ethanol, 40 minutes, room temperature;
80% ethanol, 60 minutes, room temperature;
70% ethanol, overnight, 4°C;
95% ethanol, 60 minutes, room temperature;
95% ethanol, 60 minutes, room temperature;
100% ethanol, 60 minutes, room temperature;
100% ethanol, 60 minutes, room temperature;
100% ethanol, 10 minutes, room temperature;
xylene, 120 minutes, room temperature;
xylene, 90 minutes, room temperature;
xylene, 30 minutes, room temperature;
Paraplast/Xylene (50:50), 60 minutes, room temperature;
Paraplast/Xylene (50:50), 60 minutes, room temperature;
Paraplast, 60 minutes, room temperature;
Paraplast, overnight, 58°C.
Paraplast, 60 minutes, 58°C.

Samples were then embedded in fresh Paraplast. Embedded samples were cooled at 4°C for at least 3 hours before being sectioned.

Results

Verification of morpholino knock-down and germ cell removal. We performed several tests in adult males from all three treatments (GLF, IC NIC) for the removal of the germ cells. In a first test, we set up males with wild-type AB zebrafish females for natural spawning and monitored the number of females that laid eggs, the number of clutches where at least one egg developed into embryos. In the GLF males, 17 pairs out of 28 produced eggs in but none of the was fertilised (Figure S1). In comparison, in the IC males, 13 out of 20 pairs produced eggs all of which were fertilised and in the NIC males, nine out of 20 pairs produced eggs, and all were fertilised (Figure S1).

We attempted to collect ejaculates from 62 GLF males under anaesthesia (a standard procedure in our lab), successfully collected ejaculates form 41 of the GLF males and found sperm in none of the resulting ejaculates under the light microscope at 40x magnification.

Finally, we dissected adult males from all three treatments for their testes to test for the expression of the germ cell-specific gene *piwil1*. The testes were flash frozen in liquid nitrogen for later RNA extractions. Total RNA was isolated from frozen testes using a phenol-chloroform protocol. DNAse treatment was performed using TURBO DNA-free Kit according to the manufacturer instructions (ThermoFisher, U.K.). Reverse transcription and PCR amplification were performed from 0.3µg total RNA with OneStep RT-PCR Kit following the manufacturer's instructions (QIAGEN, U.K.). Relative quantification of the B-actin and Ziwi-transcript levels was

performed with B-actin (Wang and Orban, 2007) and Ziwi specific primers (primer sequences: 5' CCAGGTTCTTCTCGTTAGCCAT 3' and 5' CGGTTAGACCAGTGAGGTAGCA 3' resulting in amplification products of 209 bp and 107 bp, respectively. The expression level of *B-actin* is constant in most tissue and therefore was used as an internal RT-PCR standard.
RT-PCR reactions were performed in Gene Amp PCR System 9700 (Applied Biosystems, U.S.) under the following conditions: 30 minutes at 50° C and 15 minutes at 95° C for reverse transcription and inactivation of reverse transcriptases, respectively. Afterwards, 40 seconds at 94° C, then 50 seconds at 50° C and 1 minute at 72° C (30 cycles), 10 minutes at 72° C (final extension step) in 50 µl reaction volume. After 30 cycles of amplification 5µl of PCR product were taken for analysis, to which 1µl of loading dye (Invitrogen, U.K.) was added. The reaction was loaded on a 2.5% agarose gel and run for 35 minutes (120V). Signals were detected and quantified with BioDoc-It Imaging System using UVP TS software.
While all samples showed the presence of the control gene *Beta actin*, we found evidence for *piwil1* expression in seven out of 11 IC males, in nine out of ten NIC males and in zero out of eight GLF males (Figure S2A). The lack of signal for *piwil1* in IC and NIC males is due to a suboptimal match of the relatively short primers. We used a different set of primers for *piwil1* (5' CTCAGATGGTGGTGGTGATCT and 3' ACGGTCACACTGTTCCTTCAG) in an additional three males per treatment and also tested for the somatic gonad specific gene *amh* (primers: 5' AGGTCAACCCGCTATCAGAAT and 3' CTGCTGTCTCTGAGGGAACAC) to verify that the tissue collected were gonads. We obtained signals for *amh* in all males except for one NIC and a signal for *piwil1* in IC and NIC males (except for one NIC male, the same that failed for *amh*) but in none of the GLF males (Figure S2B). We therefore never detected a signal for *piwil1* in any of the 11 GLF males tested.

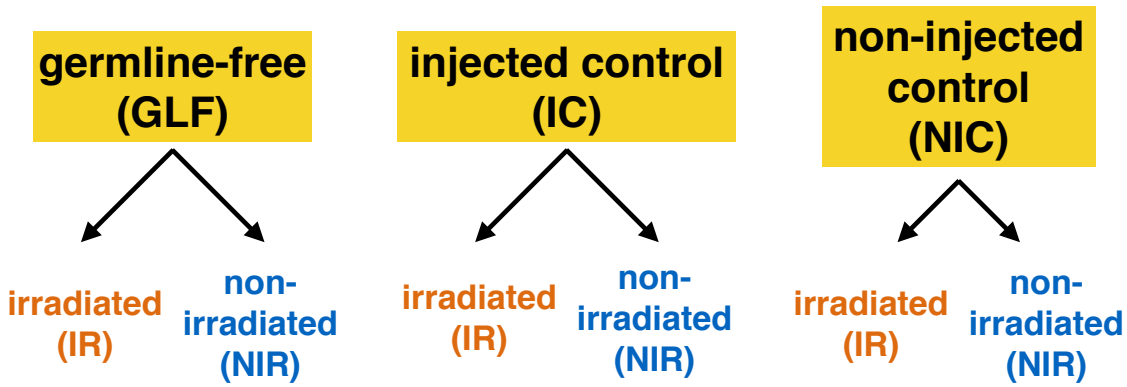


Fig. S1: Experimental design with three treatments (GLF, IC, NIC) and two sub-treatments (IR, NIR). The design used a fully factorial split-clutch design where siblings from one family were split into six sub-groups and each exposed to a unique combination of treatment and subtreatment.

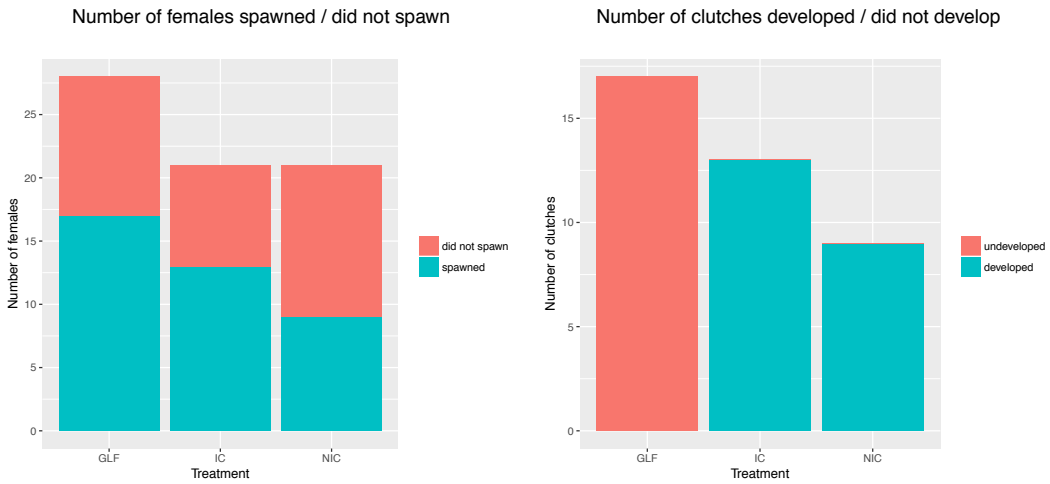


Fig. S2. Number of females set up for spawning with each of three types of males (NIC, IC and GLF) and number of clutches tested for developing embryos.

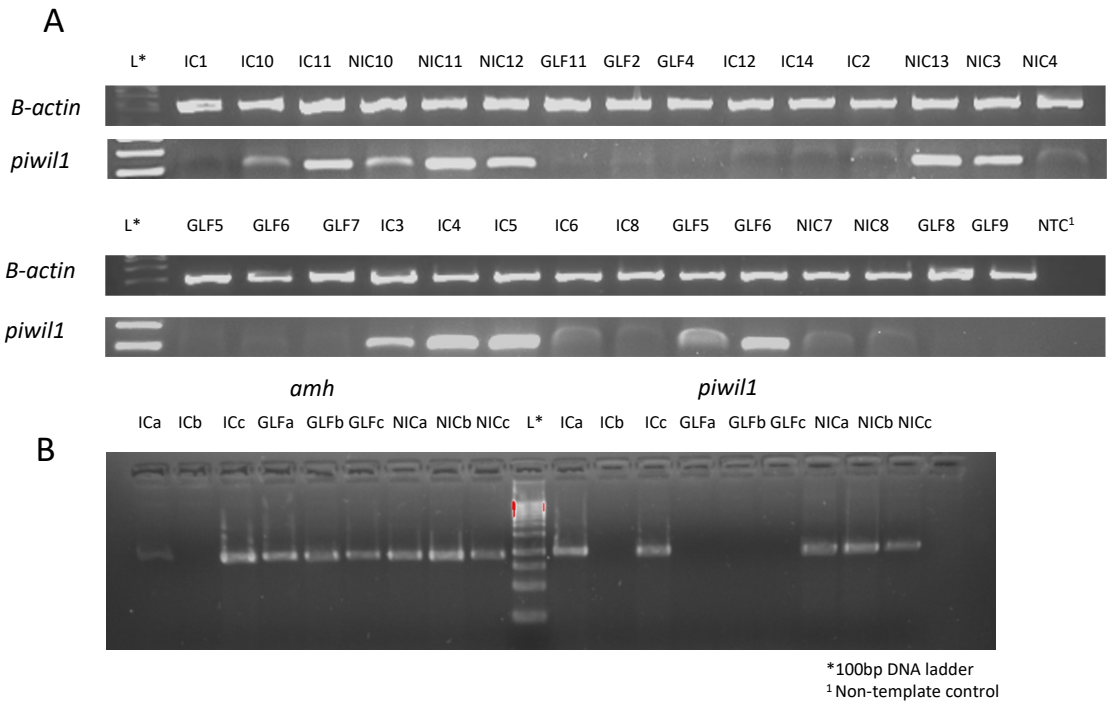


Fig. S3. PCR products for *Beta actin*, *piwil1* and *amh*. A) First run and B) second run with different set of primers for *piwil1*.

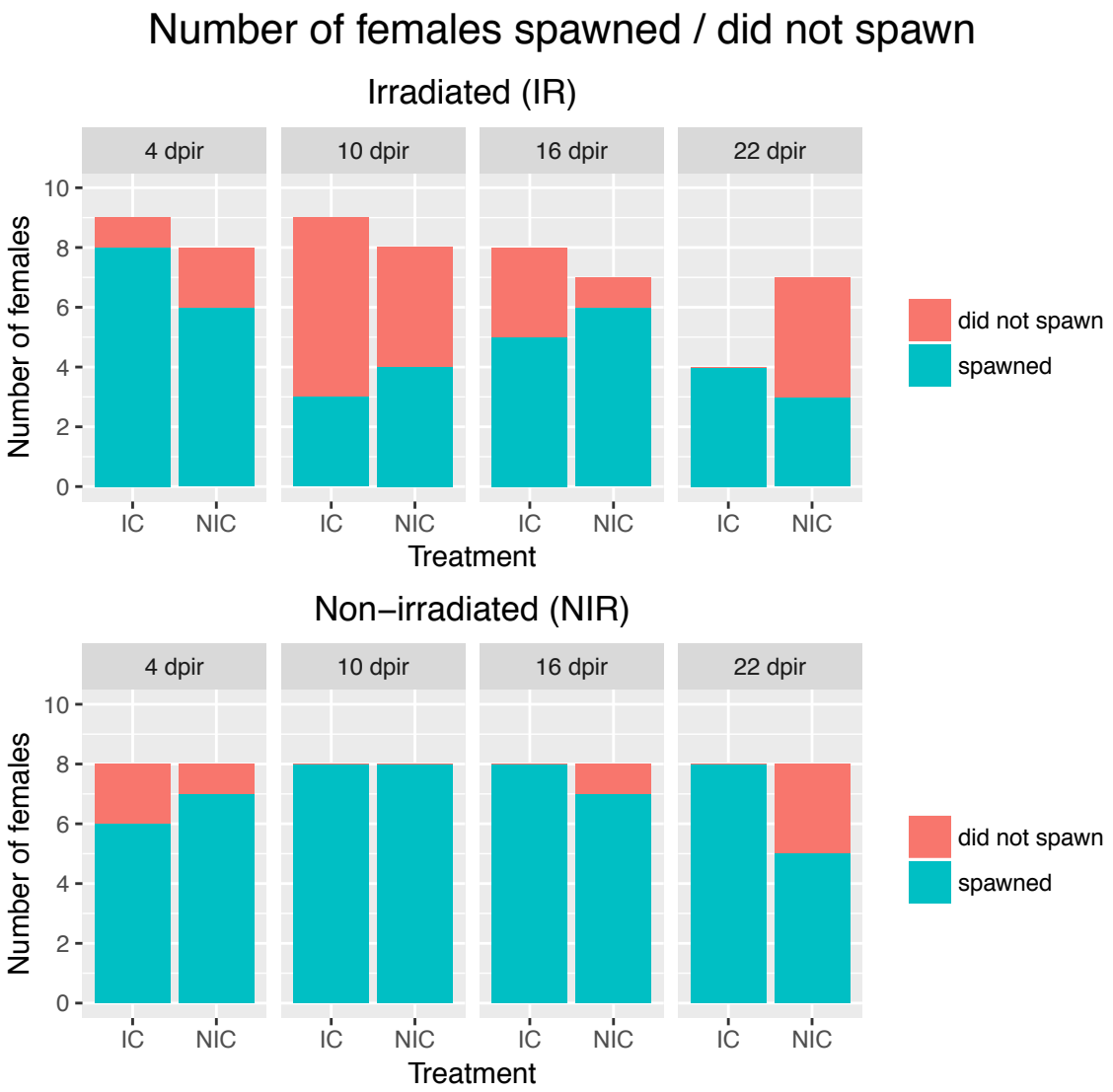


Fig. S4: Numbers of females that did spawn and did not spawn with males exposed to fours combinations of treatments (injected control: IC, non-injected control: NIC) and irradiation. Total numbers of females indicate total numbers of spawning assays performed.

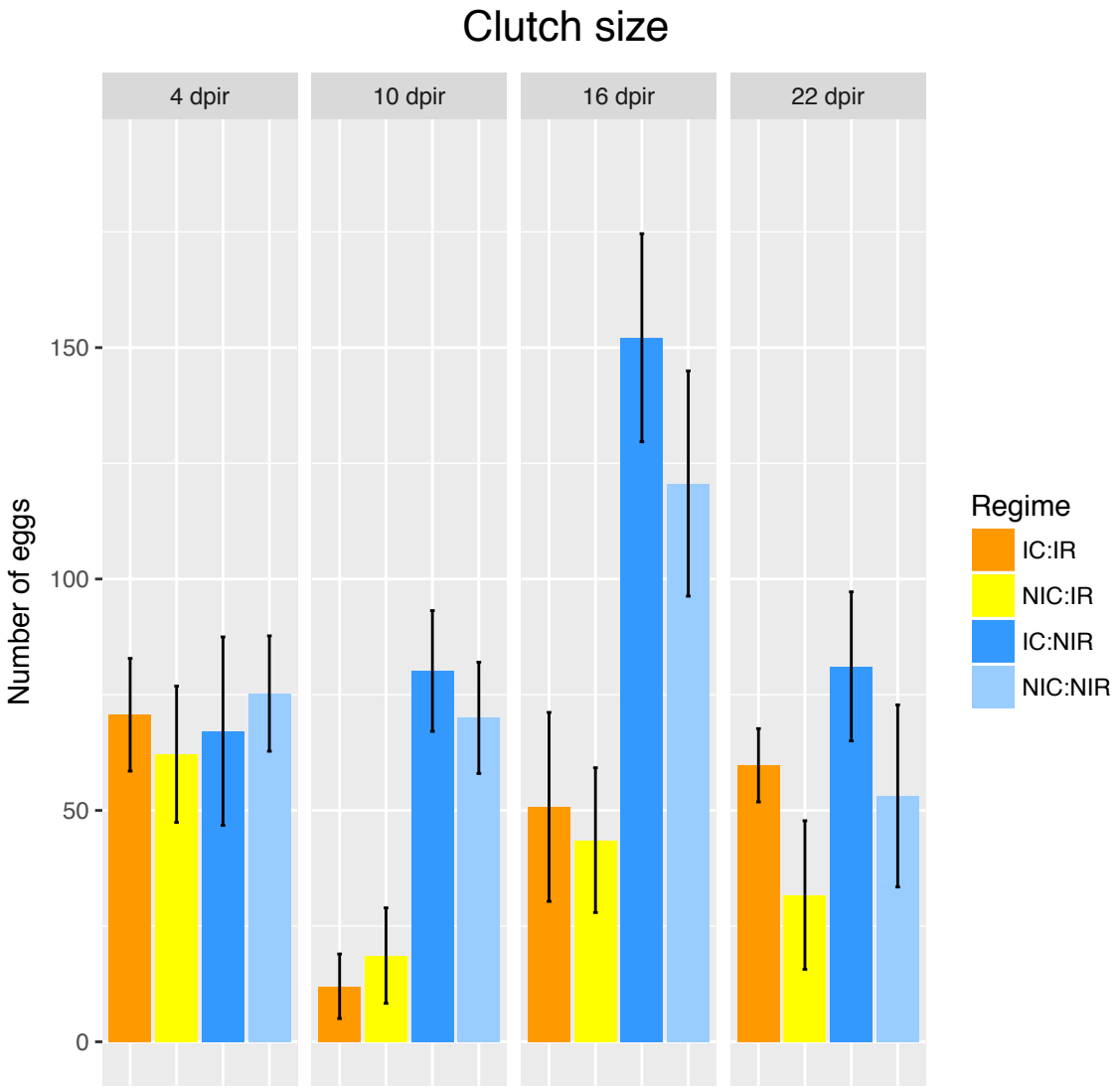


Fig. S5: Clutch sizes (total number of eggs) shown as mean ± standard error in each of the four combinations between treatment (injected control: IC, non-injected control: NIC) and irradiation (irradiated: IR, non-irradiated: NIR) from 4 days post irradiation (dpir) to 20 dpir.

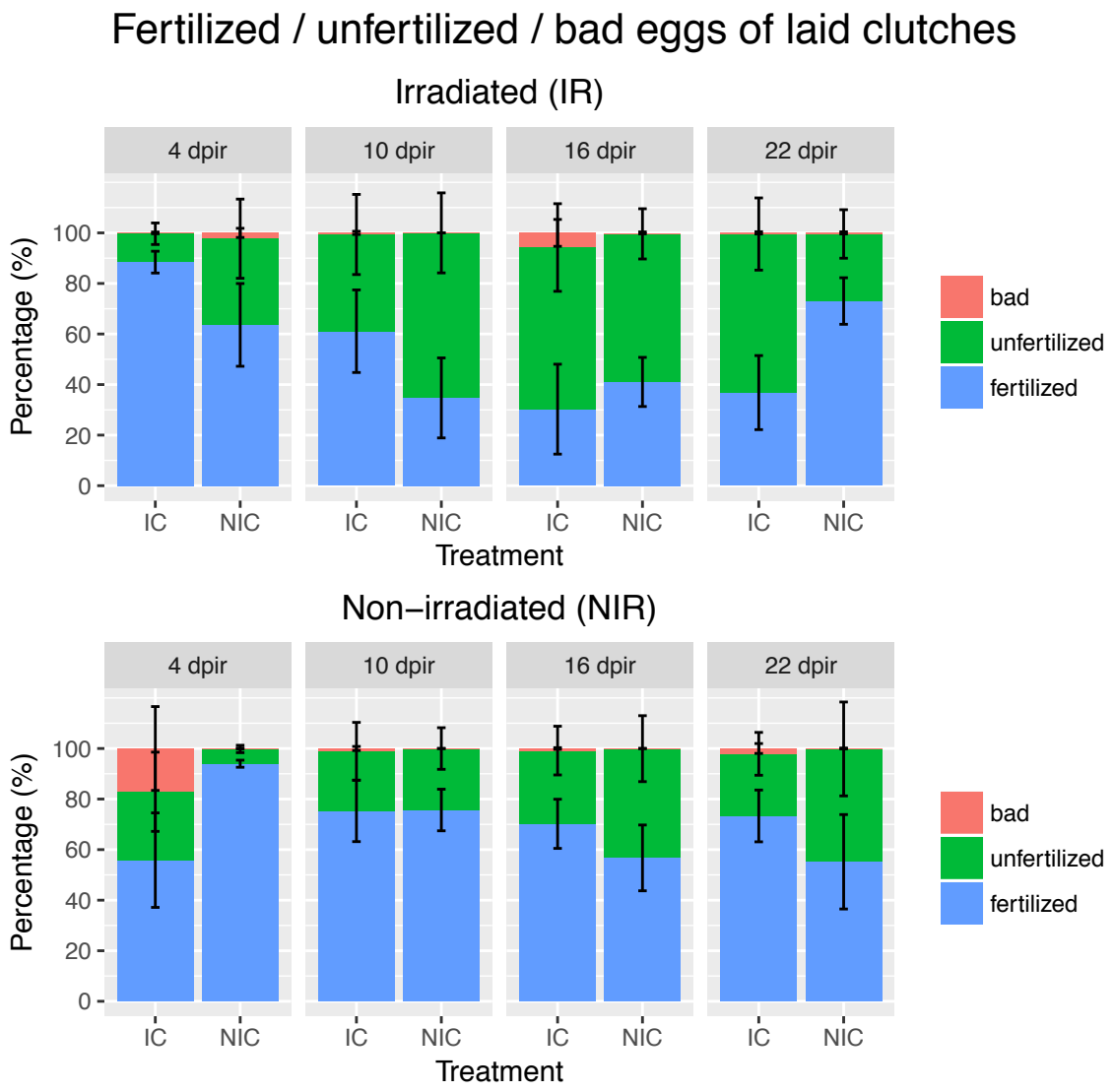


Fig. S6: Percentage of fertilized, unfertilized and bad eggs in the clutches laid by males in two different treatments of germline-carrying fish (injected control: IC, non-injected control: NIC) and sub-treatments (irradiated: IR; non-irradiated: NIR) from 4 to 22 dpir. Upper panel shows results for irradiated fish with a clear detrimental effect of irradiation on embryo survival and development. In the lower panel, the males of both treatments bred at the same time points without irradiation. Values displayed represent mean ± standard error.

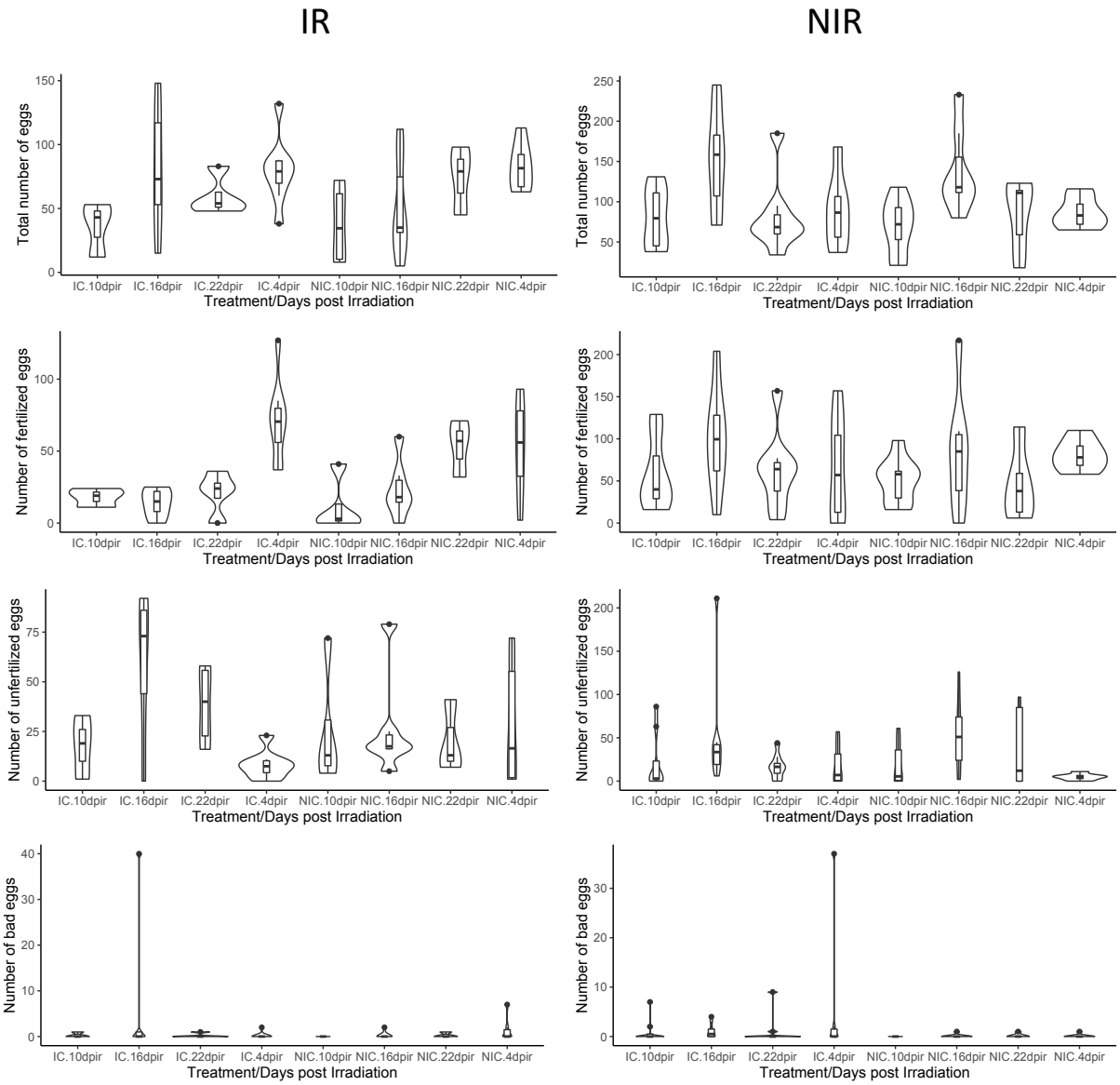


Fig. S7: Combined violin and boxplot showing total number of eggs per clutch, number of fertilized eggs, number of unfertilized eggs and the number of bad eggs in each clutch laid by females in natural spawnings with males exposed to two different treatments (injected control: IC, non-injected control: NIC) and sub-treatments (irradiated: IR; non-irradiated: NIR) at 10 to 22 days post irradiation (dpir).

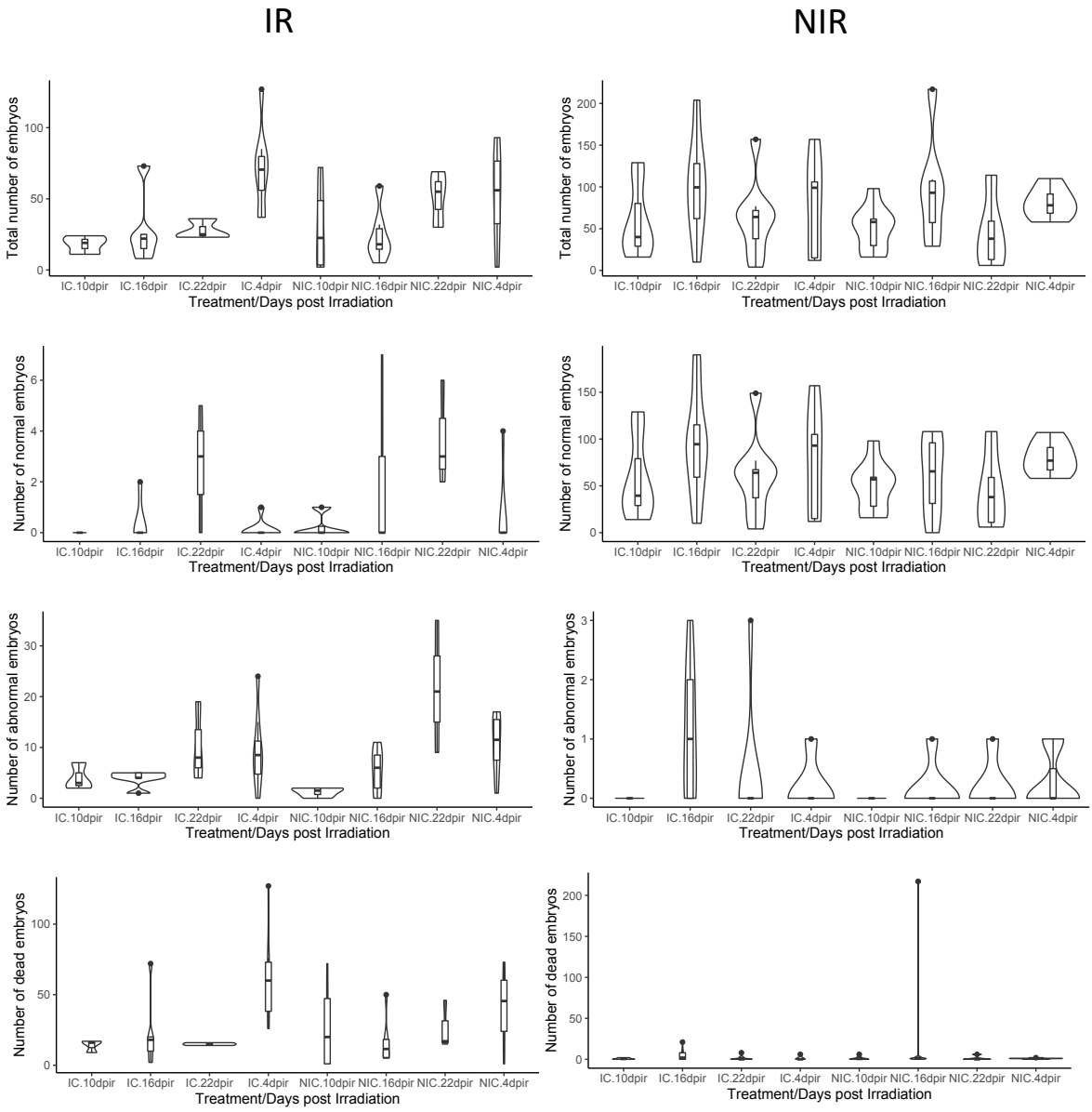
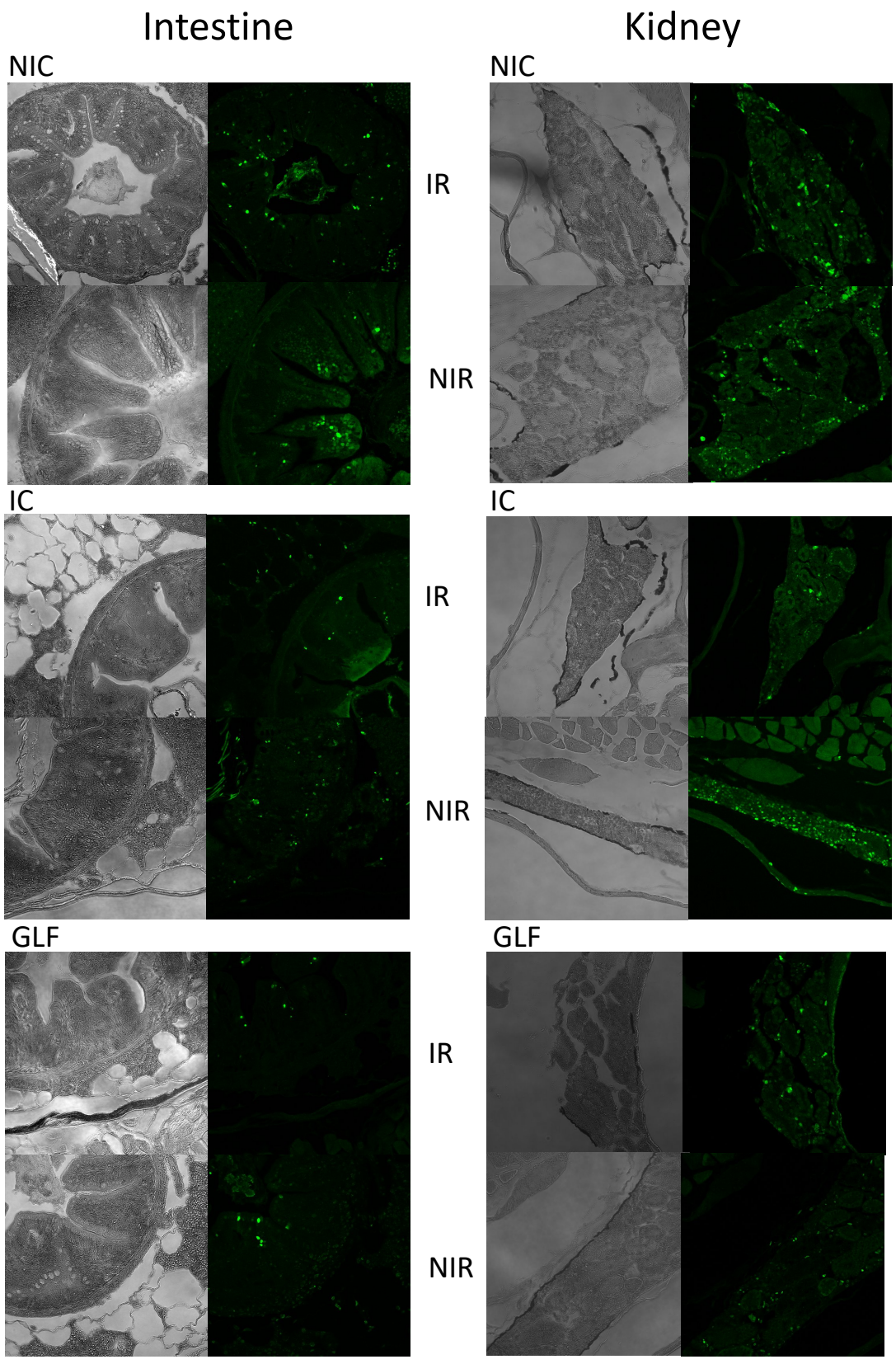


Fig. S8: Combined violin and boxplot showing total number of embryos per clutch, number of normal embryos, number of abnormal embryos and the number of dead embryos in each clutch laid by females in natural spawnings with males exposed to two different treatments (injected control: IC, non-injected control: NIC) and sub-treatments (irradiated: IR; non-irradiated: NIR) at 10 to 22 days post irradiation (dpir).


Intestine
Kidney
NIC
NIC
IR
NIR
IC
IC
IR
NIR
GLF
GLF
IR
NIR

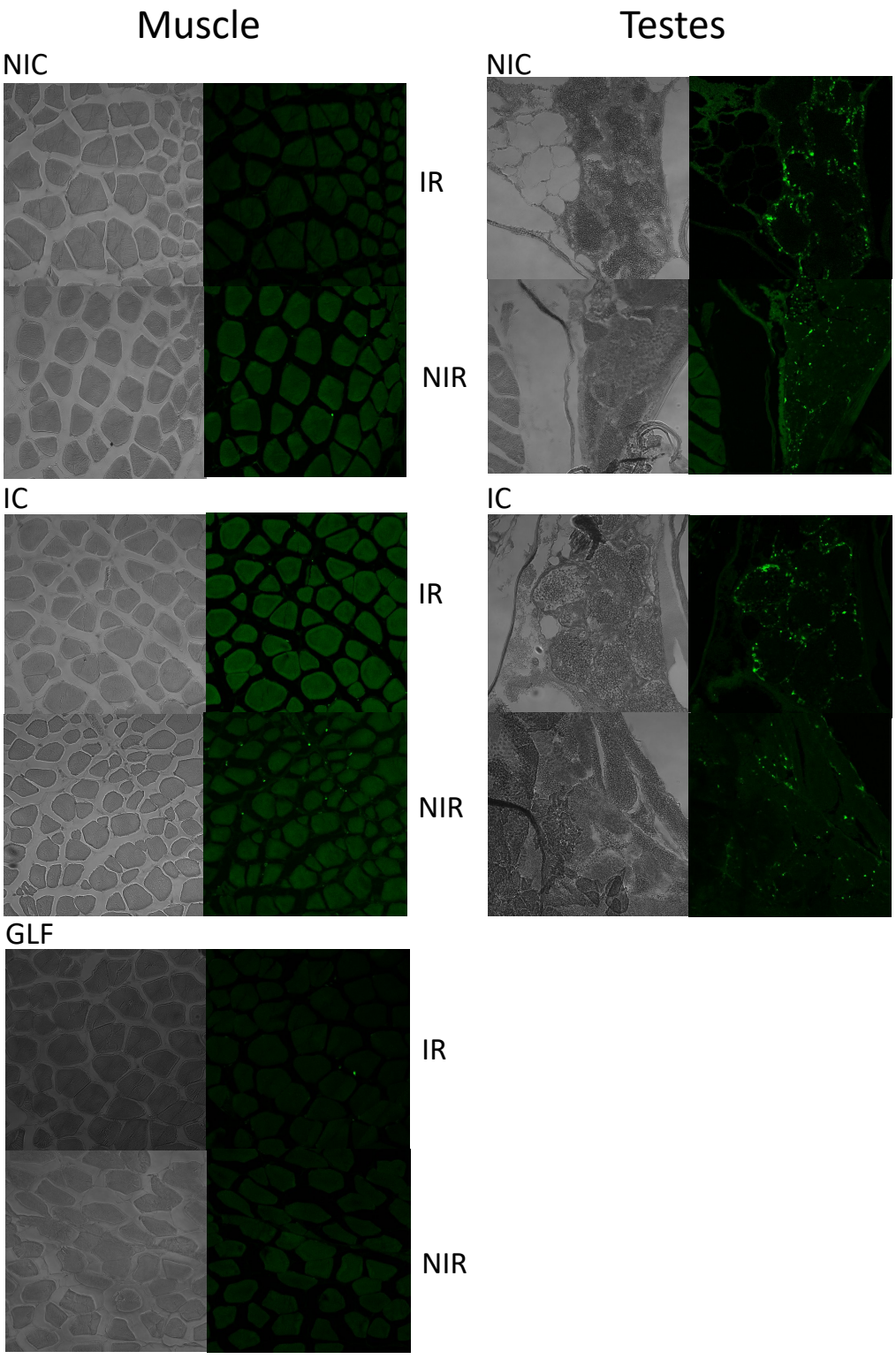


Fig. S9: Histology images of the four tissues (Intestine, Kidney, Muscle, Testes) assessed with a TUNEL assay, with the corresponding brightfield image and the fluorescent image next to each other. One example image is shown for each regime and tissue.

Table S1. The linear model of the effects of treatment (NIC or IC), irradiation (IR or NIR) and days-post-irradiation (dpir) on spawning.

Fixed effect	Chisq	Df	P
Treatment	0.883	1	0.347
Irradiation	10.519	1	0.001
dpir	2.801	3	0.423
Treatment*Irradiation	0.004	1	0.948
Treatment*dpir	8.689	3	0.034
Irradiation*dpir	9.626	3	0.022

Table S2. The generalized linear mixed-effects model of the effects of treatment (NIC or IC), irradiation (IR or NIR) and days-post-irradiation (dpir) on clutch size.

Fixed effect	Chisq	Df	P
Treatment	62.815	1	< 0.001
Irradiation	655.614	1	< 0.001
dpir	23.599	3	< 0.001
Treatment*Irradiation	0.795	1	0.373
Treatment*dpir	69.989	3	< 0.001
Irradiation*dpir	483.362	3	< 0.001
Treatment*Irradiation*dpir	41.895	3	< 0.001

Table S3. The generalized linear mixed-effects model of the effects of treatment (NIC or IC), irradiation (IR or NIR) and days-post-irradiation (dpir) on fertilization (i.e. number of fertilized eggs in a clutch).

Fixed effect	Chisq	Df	Pr
Treatment	1.875	1	0.171
Irradiation	213.280	1	< 0.001
dpir	7.626	3	0.054
Treatment*Irradiation	0.336	1	0.562
Treatment*dpir	85.099	3	< 0.001
Irradiation*dpir	72.977	3	< 0.001
Treatment*Irradiation*dpir	398.394	3	< 0.001

Table S4. The generalized linear mixed-effects model of the effects of treatment (NIC or IC), irradiation (IR or NIR) and days-post-irradiation (dpir) on embryo development (i.e. number of normally developing embryos in a fertilized clutch).

Fixed effect	Chisq	Df	Pr
Treatment	73.209	1	< 0.001
Irradiation	525.285	1	< 0.001
dpir	4.065	3	0.255
Treatment*Irradiation	29.794	1	< 0.001
Treatment*dpir	27.864	3	< 0.001
Irradiation*dpir	26.407	3	< 0.001
Treatment*Irradiation*dpir	8.437	3	0.038