

# SUPPLEMENTARY INFORMATION

## Accurate quantification of homologous recombination in zebrafish: brca2 deficiency as a paradigm

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**Supplementary Table 1: Mutation and primers used for genotyping**

Line	Mutation	Forward	Reverse
<i>brca2</i> <sup>hg5</sup>	<i>c.1972C&gt;T</i> p.Q658X exon 11	5'-CAAGTCAACGTCTATCACAACC-3'	5'-TGATGACTCTGAACTTCTCCC-3'
<i>brca2</i> <sup>sa22682</sup>	<i>c.4006C&gt;T</i> p.Q1336X exon 11	5'-TCTAGCTCCTCAGGGAAGCA-3'	5'-AGGTTTCATGGTCCTTTGCAG-3'
<i>brca2</i> <sup>cmg35</sup>	<i>c.534del13</i> p.V178fs exon 8	5'-TGTAGTAGGCTAAGCAAATACGG-3'	5'-CAGAGTCCTTTGAGAGAGATGG-3'

**Supplementary Table 2: PCR reaction mix**

PCR component	volume/reaction (μl)
Kapa2G Robust MM (2x)	10
Forward primer (10μM)	0.6
Reverse primer (10μM)	0.6
H <sub>2</sub> O	7.8
Dissolved G-block	1
Total volume/PCR reaction	20

**Supplementary Table 3: PCR primers**

Forward primer	5'-CCGCTAGCTAATACGACTCACTA-3'
Reverse primer	5'-AAAAGCACCGACTCGGTG-3'

**Supplementary Table 4: PCR program**

94° C	4 min	
94° C	30 sec	6 X (touchdown)
58° C	30 sec	
72° C	1 min	
94° C	40 sec	25 X
52° C	40 sec	
72° C	30 sec	
72° C	10 min	

**Supplementary Table 5: Time kinetics experiment**

	Mean number of Rad51 foci/cell		
	10Gy (95% CI)	20Gy (95% CI)	0Gy (95% CI)
1h	4.49 ± 0.81	4.71 ± 1.2	
3h	4.57 ± 0.85	4.68 ± 0.87	
5h	3.87 ± 0.99	5.41 ± 1.47	0.54 ± 0.21
7h	3.88 ± 0.56	6.33 ± 2.56	
9h	3.23 ± 0.8	5.64 ± 1.49	0.4 ± 0.2

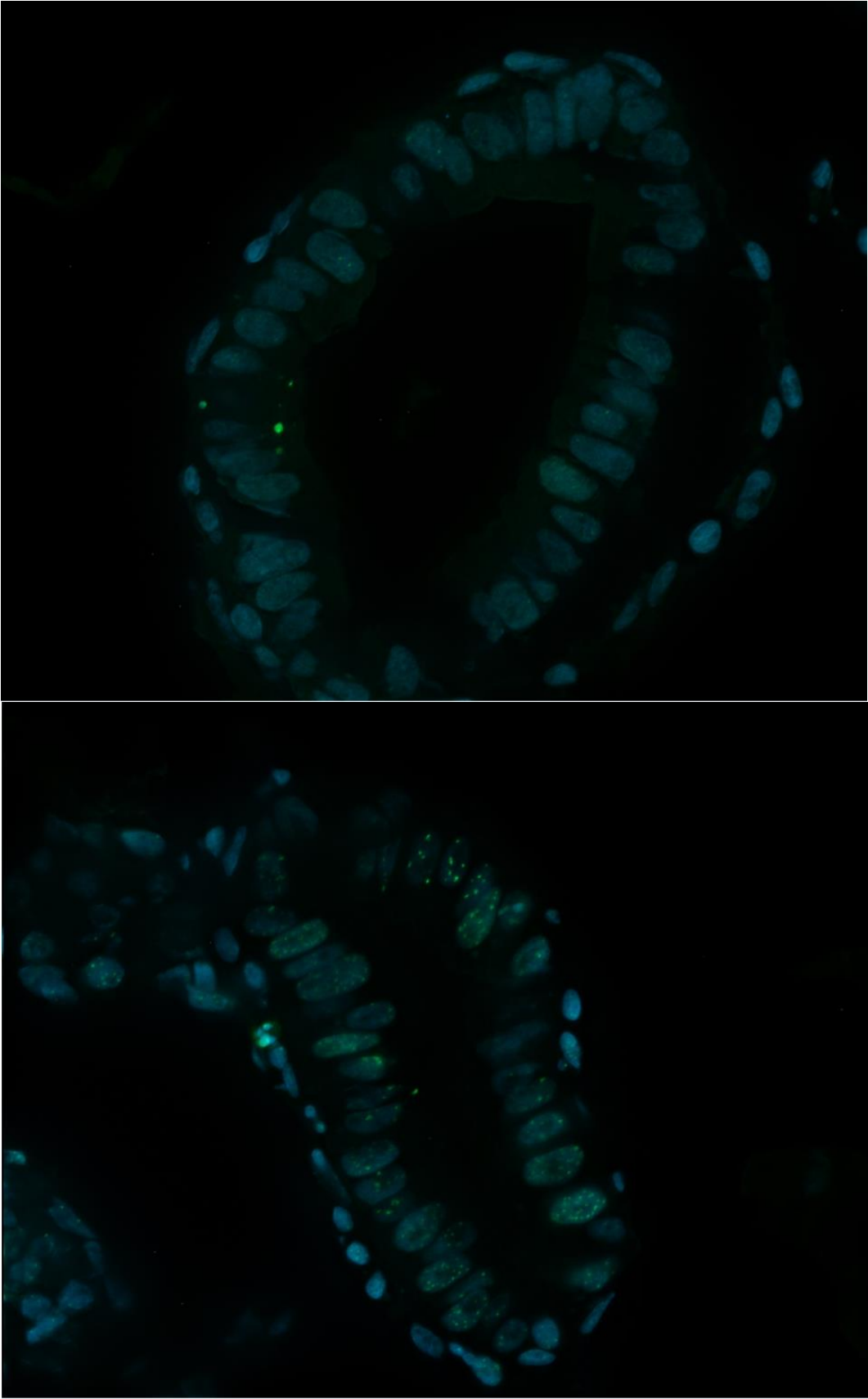
**Supplementary Table 6: Comparison of quantification**

Dose	Hypothesised value (95% CI)	True value (95% CI)
20Gy	6.14 ± 1.36	6.06 ± 1.35
0Gy	0.67 ± 0.07	0.47 ± 0.04

**Supplementary Table 7: Comparing the amount of Rad51 foci/cell between different genotypes for three mutations and combined**

	Mean number of Rad51 foci/cell			
	<i>brca2</i> <sup>hg5</sup>	<i>brca2</i> <sup>sa22682</sup>	<i>brca2</i> <sup>cmg35</sup>	Combined
<i>brca2</i> <sup>+/+</sup>	5.58 ± 0.83	6.1 ± 0.22	6.05 ± 0.81	5.70 ± 0.39
<i>brca2</i> <sup>+/-</sup>	5.09 ± 0.72	5.2 ± 0.76	4.39 ± 0.59	4.95 ± 0.43
<i>brca2</i> <sup>-/-</sup>	0.18 ± 0.14	0.12 ± 0.08	0.13 ± 0.06	0.15 ± 0.05

**Supplementary Fig S1:  $\gamma$ H2AX staining.** Unirradiated (upper) and irradiated (lower) gut section of 72 hpf embryo. Dose administered was 20 Gy, followed by fixation at 5 hpi.



**Supplementary Fig S5: Result of comparing quantification methods.** Foci were either quantified according to the method used in the time kinetics experiment (hypothesised value) or by counting the mean number of foci in the geminin positive cells (true value). Values show Rad51 foci/cell  $\pm$  95% CI.

Comparison of quantification

