Supplementary Figure Legends

Figure S1. *Rad51* mutant fish are all infertile males. (A) H&E stained histological sections of 4 mpf wild type and mutant kidneys using a 20X objective. The arrow points out the area where mature spermatozoa reside. Scale bar=100 μm. (B) Capillaries used to obtain sperm from wild type and mutant fish. Numbers indicate the number of fish giving sperm among the fish tested. Two-tailed Fisher's exact test, *P*=.0002. +/+, n=9. -/- n=13.

Figure S2. Loss of *rad51* causes DNA damage sensitivity. (A) Chromosome spreads of 24 hpf wild type and mutant embryos treated with 5 μg/ml mitomycin C for 20 hours. White arrows indicate premature chromatid separation events (i). Quantification of the damage (ii). Mann-Whitney test, *P*=.001, $n_{+/+}$=16, $n_{-/-}$=19. (B) Chromosome spreads of 24 hpf wild type and mutant embryos treated with 1 nM CPT for 20 hours. White arrows indicate breaks and radial structures in response to topoisomerase inhibition (i). Quantification of the damage (ii). Mann-Whitney test, *P*<.0001, $n_{+/+}$=12, $n_{-/-}$=17. (C) Chromosome spreads of 24 hpf wild type and mutant embryos treated with 10μ μM DiQ for 20 hours (i). Quantification of the damage (ii). Mann-Whitney test, *P*=.13, $n_{+/+}$=14, $n_{-/-}$=29. Bars represent mean +/- SEM in all graphs.

Figure S3. NHEJ inhibition does not lead to synthetic lethality in *rad51* mutants. Morphology of 24 hpf embryos treated with varying concentrations of SCR-7 for 20 hours.

Figure S4. ***Rad51* mutant fish are smaller than their wild type siblings.** (A) Representative images of mutant and wt fish at 23 dpf (i). (ii) Graph showing difference in size between the genotypes. Two-sided Student's t-test (*P*=.0016), $n_{+/+}$=11, $n_{-/-}$=7. Scale bar=1 mm. (B) Comparison of mutant and wild type fish at 4 mpf. Two-sided Student's t-test (*P*=.004), $n_{+/+}$=28, $n_{-/-}$=19. Scale bar=1 cm. (C) (i) Representative images of mutant and wt fish at 8 mpf. (ii) Graph showing difference in size between the genotypes. Two-sided Student's t-test (*P*=.023), $n_{+/+}$=29, $n_{-/-}$=31. Scale bar=1 cm. Bars represent mean +/- SEM in all graphs.

Figure S5. *Rad51* mutants develop micropthalmia. (A) Eye size at 5 dpf. Two-sided Student's t-test (*P*=.0001), $n_{+/+}$=21, $n_{-/-}$=13. (B) Eye size at 23 dpf. Two-sided Student's t-test (*P*=.013), $n_{+/+}$=11, $n_{-/-}$=7.

Figure S6. FACS gating. (A) Gate selecting for: i) cells and excluding debris, (ii) singlets, (iii) and (iv) viable cells based on propidium-iodide staining. (B) Gating for apoptotic cells using AnnexinIV-GFP (AV-GFP) antibody and PI staining. (i) Gating set on an unstained control, (ii) gating on an AV-GFP and PI-stained control. Viable cells are in the bottom left, apoptotic cells in the top left, dead cells in the top right and necrotic cells in the bottom right. (C) Gating for *gata1*:GFP-positive cells. (i) Gating set on a non-transgenic fish, (ii) same gating on a transgenic fish. (D) Gating for *cd41:GFP*-positive cells. (i) Gating set on a non-transgenic fish, (ii) same gating on a transgenic fish, iii) GFP+ cells are split into GFP^{dim} (thrombocytic progenitors) and GFP^{high} (mature thrombocytes) by selecting for two equally-sized gates at the 50% fluorescence mark. (E) Gating for different cell lineages based on the FSC and SSC parameters. (F) Gating for BrdU+ cells on fixed cells. (i) Selection for cells, (ii) singlets, (iii) BrdU+ cells, (iv) same gating as in iii, but on a BrdU-injected sample.

Figure S7. *P53* co-mutation increases the incidence of tumor development in double mutant fish, but does not rescue their fertility or size. (A) Male and female *p53*-/- *rad51*-/- fish at 4 mpf. (B) H&E stained histological sections of 4 mpf *p53*-/- and *p53*-/- *rad51*-/- ovaries using a 10X objective. Oocytes of different maturation stages are apparent in both genotypes. Scale bar=100µm. (C) H&E stained histological sections of 4 mpf *p53*-/- and *p53*-/- *rad51*-/- testes using a 20X objective. Arrows indicate normal location of mature spermatozoa Scale bar=100µm. (D) Quantification of the size of wild type and *p53*-/- *rad51*-/- fish at 4 mpf. Two-tailed Student's t-test, P=.008, $n_{p53+/+\ rad51+/+}$=27, $n_{p53-/-\ rad51-/-}$=9. (E) 5-month-old *p53*-/- *rad51*-/- fish with tumor behind the eye (top). Accompanying H&E stained histological sections of the tumor (bottom) using 2.5 and 63X objectives. Scale bar=500 µm and 10 µm respectively. (F) (i) and (ii) Tumors observed in 8 mpf female *p53*-/- *rad51*-/- fish in the body cavity around the ovarian tissue. Scale bar=500 µm and 10 µm respectively. Overall, 3/9 aged double mutants showed tumors.

Figure S8. Inflammation marker gene expression (A) Wt fish show increased inflammation markers *il1b* (i) and *il8* (ii) six hours post injection. +/+, n=6. -/-, n=6. Two sided Student's t-test, P=.009 (i) and P=.015 (ii). (B) There is no difference in expression between wt and mutant fish in *il1b* (i) and *il8* (ii). +/+, n=6. -/- n=6. Two sided Student's t-test. (C) Relative expression of monocyte/macrophage markers *marco* (i) and *csf1r* (ii) in response to four pI:pC injections in wt fish. Two-sided Student's t-test, P<.0001 (i) and P=.0002 (ii), n_{NI}=10, n_{I}=9. Bars represent geometric mean + 95% confidence interval (CI). NI=non-injected, I=injected. (D) Injection

scheme for determining cell proliferation in pI:pC injected fish. The experiment was started with a cohort of 36 wt and 18 mutant fish. 12 wt were injected with PBS, 12 with pI:pC, 6 with PBS+BrdU and 6 with pI:pC+BrdU. 12 mutants were injected with pI:pC and 6 with pI:pC+BrdU. On the next day, the BrdU co-injected fish were culled and analysed. On day 7, 6 wt were injected with PBS, 6 with pI:pC, 6 with PBS+BrdU and 6 with pI:pC+BrdU. 6 mutants were injected with pI:pC and 6 with pI:pC+BrdU. On the next day, the BrdU co-injected fish were culled and analysed. On day 14, 6 wt fish were injected with PBS and 6 with pI:pC. 6 mutants were injected with pI:pC. On day 21, the same fish were injected again with the same substances and BrdU. All remaining fish were culled the next day and analysed.

Figure S9: Mutation in *rad51* does not lead to acetaldehyde sensitivity

(A) Schematic of the experimental design. Both wild type and *rad51*-/- fish were injected every seven days with 10 µl 1% acetaldehyde, four injections in total. All fish were culled 3 days after the last injection. Control fish were not injected; Rad51+/+, $n_{non\text{-}injected}$=9, $n_{injected}$=8; *rad51*-/-, $n_{non\text{-}injected}$=10, $n_{injected}$=9. (B) Absolute number of cells belonging to different blood lineages in the kidney gained by combining FACS data with the cell counts shown in A. Statistical tests were carried out individually for each cell type, using two-way ANOVA. *P*-value shown on the graph stems from a *post-hoc* Šidak multiple comparison test, comparing non-injected to injected fish within each genotype. For all groups, n is the same as in A. (C) The total number of cells in the kidney in injected and non-injected fish. Statistical testing was carried out using two-way ANOVA. There was a statistically significant effect of mutation status ($F(1,32)=91.2$, $P<.0001$) and of injection status ($F(1,32)=12.29$, $P=.0014$), as well as the interaction between the two ($F(1,32)=18.49$, $P=.0001$). *P*-value shown on the graph is from a *post-hoc* Tukey multiple comparison test. For all groups, n is the same as in A. (D) Viable cells as determined by PI-staining. Two-way ANOVA revealed a significant influence of mutation status ($F(1,32)=27.47$, $P<.0001$), as well as injection status ($F(1,32)=35.28$, $P<.0001$). *P*-values on the graph stem from a *post-hoc* Tukey multiple comparison test. Bars represent mean +/- SEM in B, C and D. (E) Relative gene expression of *p53.* Two-way ANOVA revealed a significant influence of mutation status ($F(1,32)=16.67$, $P=.0003$). Bars represent geometric mean + 95% CI.

Table S1. Antibodies used for western blotting and immunostaining.

Table S2. Primers used for qPCR.

Table S3. Full data on ISH staining in Figure 5A.

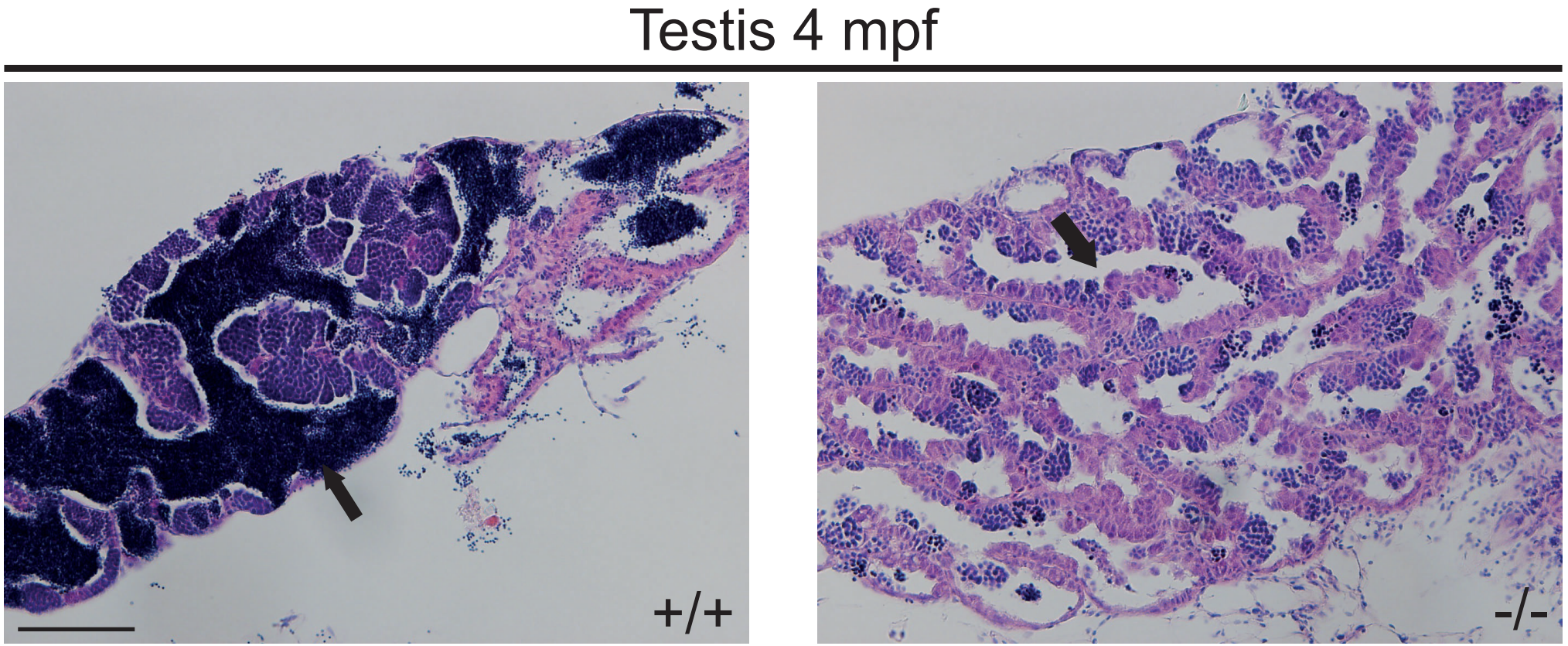
Supplementary References

1. Kyritsis N, Kizil C, Zocher S, et al. Acute inflammation initiates the regenerative response in the adult zebrafish brain. *Science*. 2012;338(6112):1353–1356.

2. Wittamer V, Bertrand JY, Gutschow PW, Traver D. Characterization of the mononuclear phagocyte system in zebrafish. *Blood*. 2011;117(26):7126–7135.

Figure S1

A



B

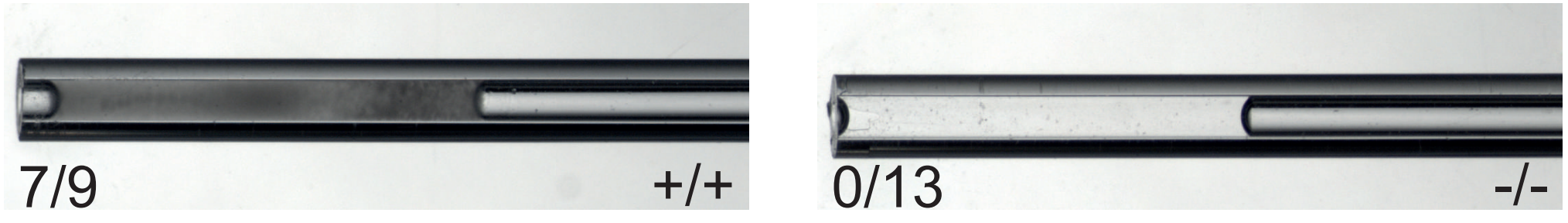


Figure S2

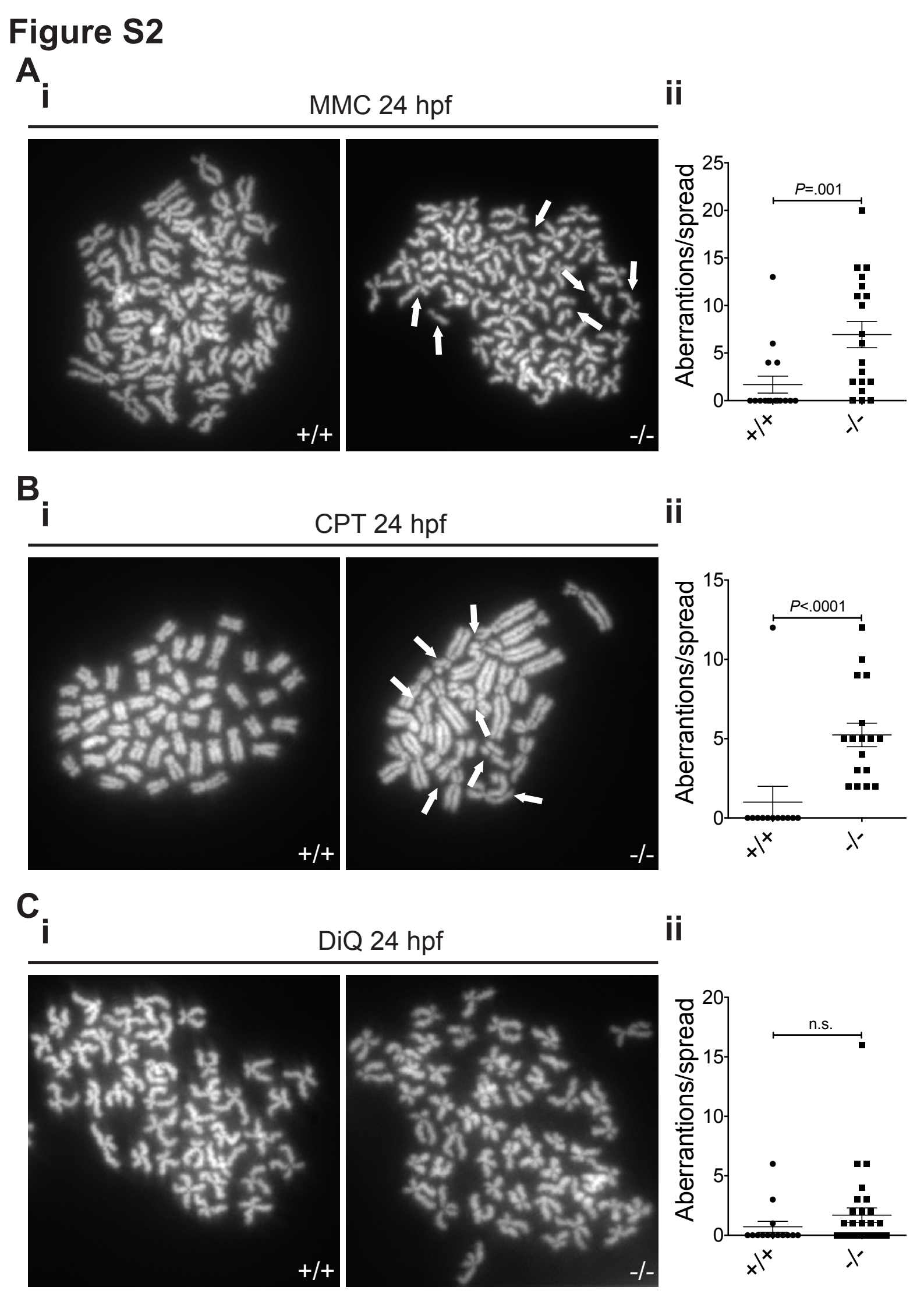

A
i
MMC 24 hpf
+/+
-/-
ii
Aberrantions/spread
P=.001
+/+
-/-
B
i
CPT 24 hpf
+/+
-/-
ii
Aberrantions/spread
P<.0001
+/+
-/-
C
i
DiQ 24 hpf
+/+
-/-
ii
Aberrantions/spread
n.s.
+/+
-/-

Figure S3

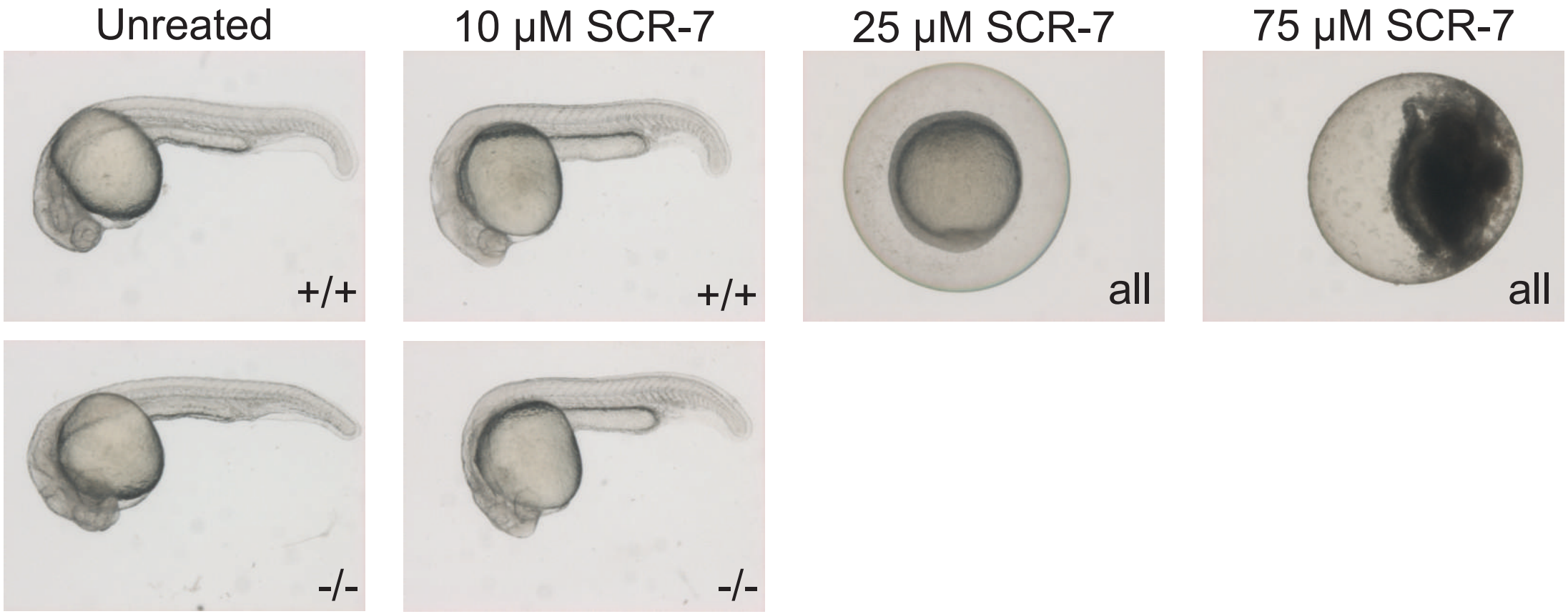
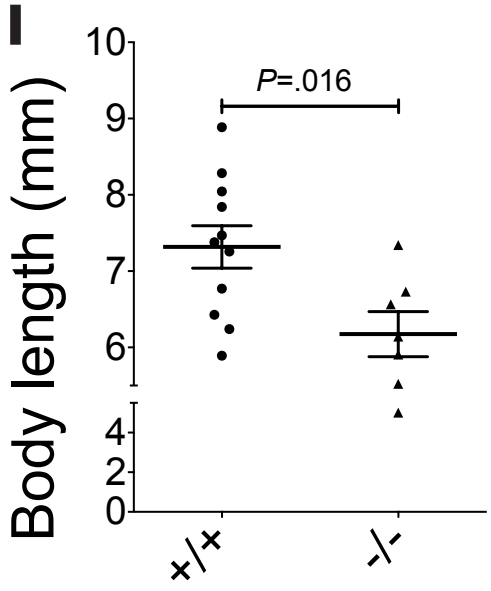


Figure S4

A

i

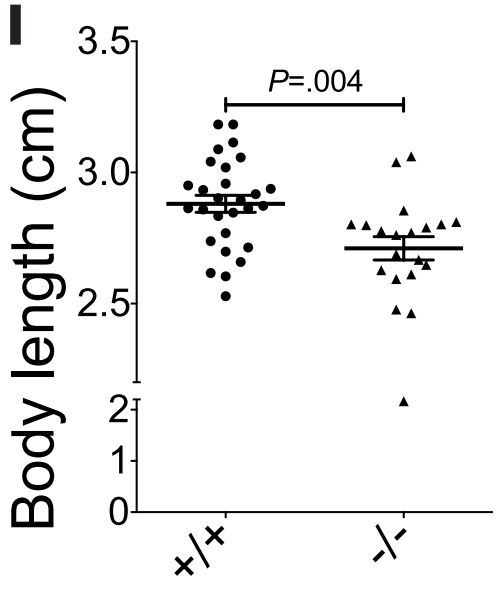
ii



B

i

ii



C

i

ii

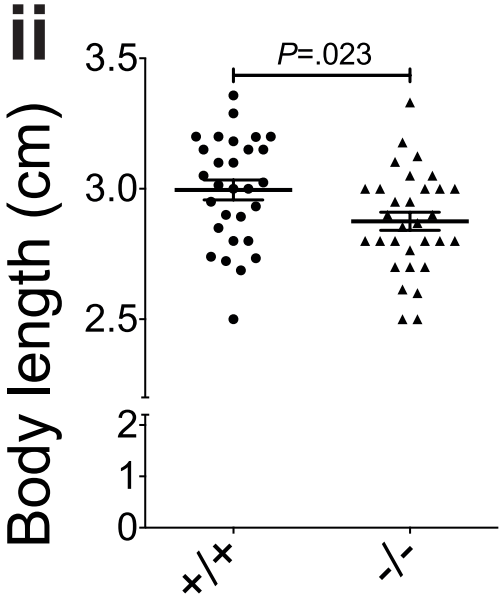


Figure S5

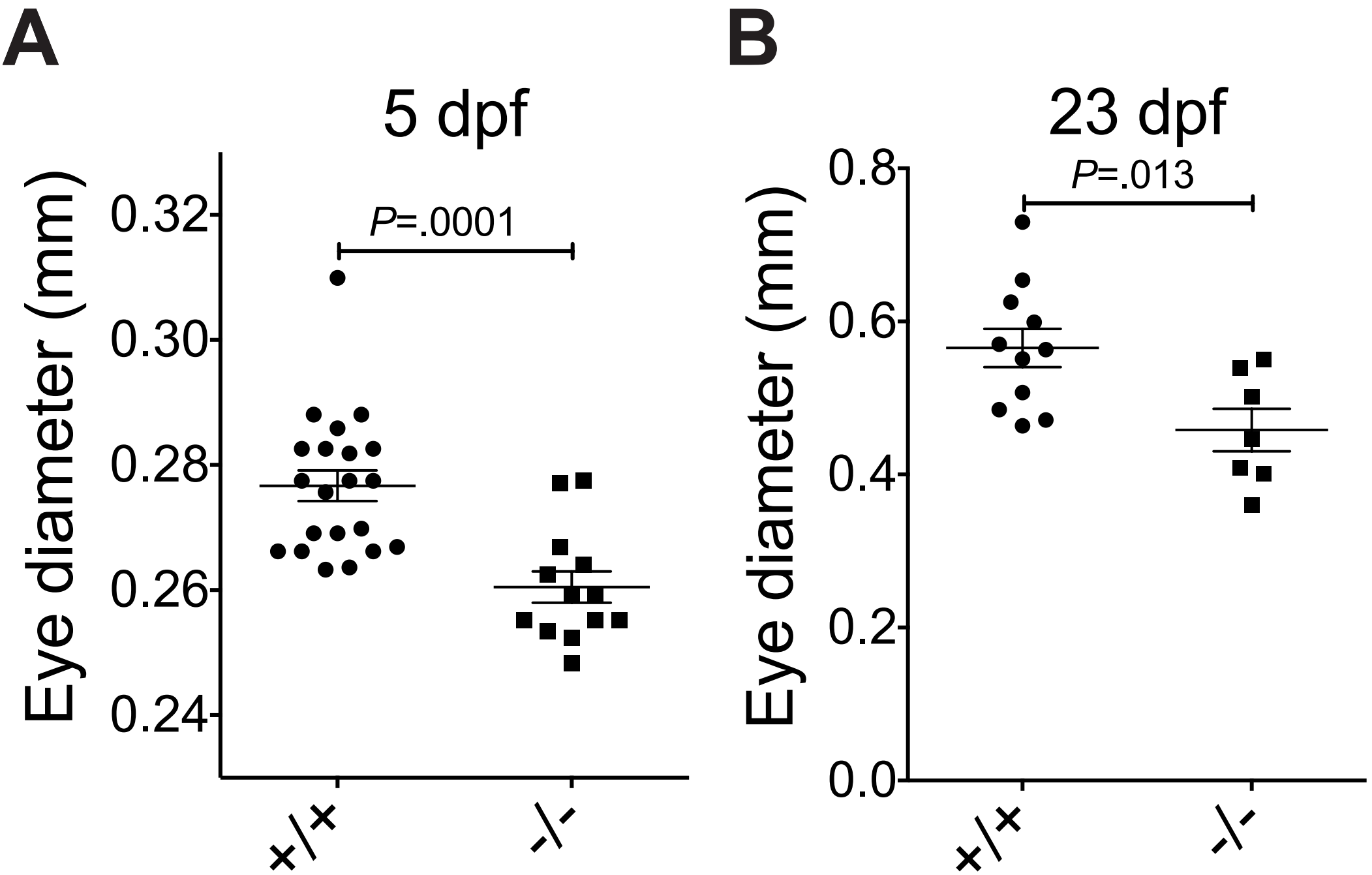

A
5 dpf
Eye diameter (mm)
0.32
0.30
0.28
0.26
0.24
P=.0001
+/+
-/-
B
23 dpf
Eye diameter (mm)
0.8
0.6
0.4
0.2
0.0
P=.013
+/+
-/-

Figure S6

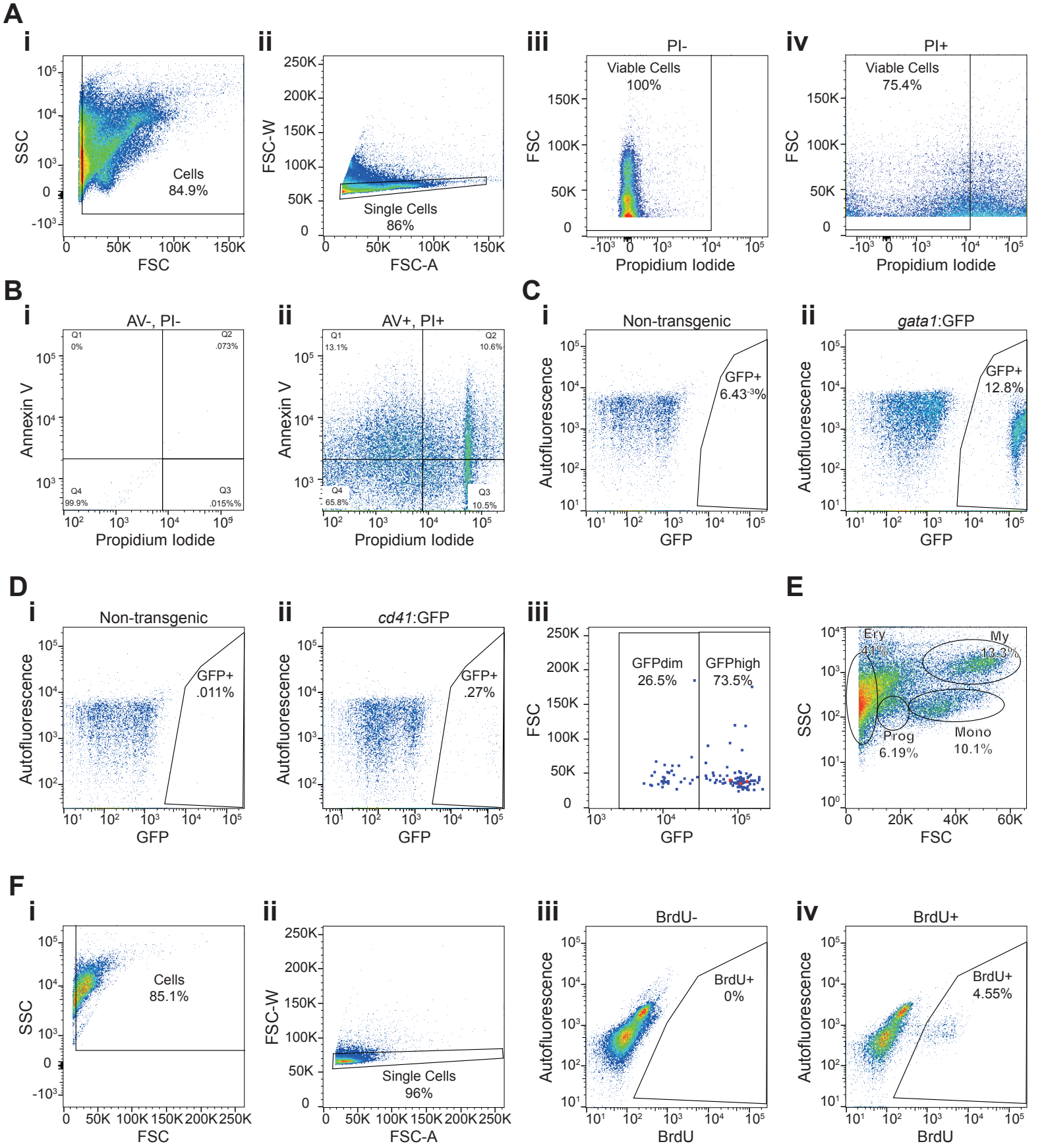

A
i
SSC
FSC
Cells
84.9%
ii
FSC-W
FSC-A
Single Cells
86%
iii
PI-
Viable Cells
100%
FSC
Propidium Iodide
iv
PI+
Viable Cells
75.4%
FSC
Propidium Iodide
B
i
AV-, PI-
Q1
0%
Q2
.073%
Q4
99.9%
Q3
.015%%
Annexin V
Propidium Iodide
ii
AV+, PI+
Q1
13.1%
Q2
10.6%
Q4
65.8%
Q3
10.5%
Annexin V
Propidium Iodide
C
i
Non-transgenic
GFP+
6.43-3%
Autofluorescence
GFP
ii
gata1:GFP
GFP+
12.8%
Autofluorescence
GFP
D
i
Non-transgenic
GFP+
.011%
Autofluorescence
GFP
ii
cd41:GFP
GFP+
.27%
Autofluorescence
GFP
iii
GFPdim
26.5%
GFPhigh
73.5%
FSC
GFP
E
Ery
41%
My
13.3%
Prog
6.19%
Mono
10.1%
SSC
FSC
F
i
Cells
85.1%
SSC
FSC
ii
Single Cells
96%
FSC-W
FSC-A
iii
BrdU-
BrdU+
0%
Autofluorescence
BrdU
iv
BrdU+
BrdU+
4.55%
Autofluorescence
BrdU

Figure S7

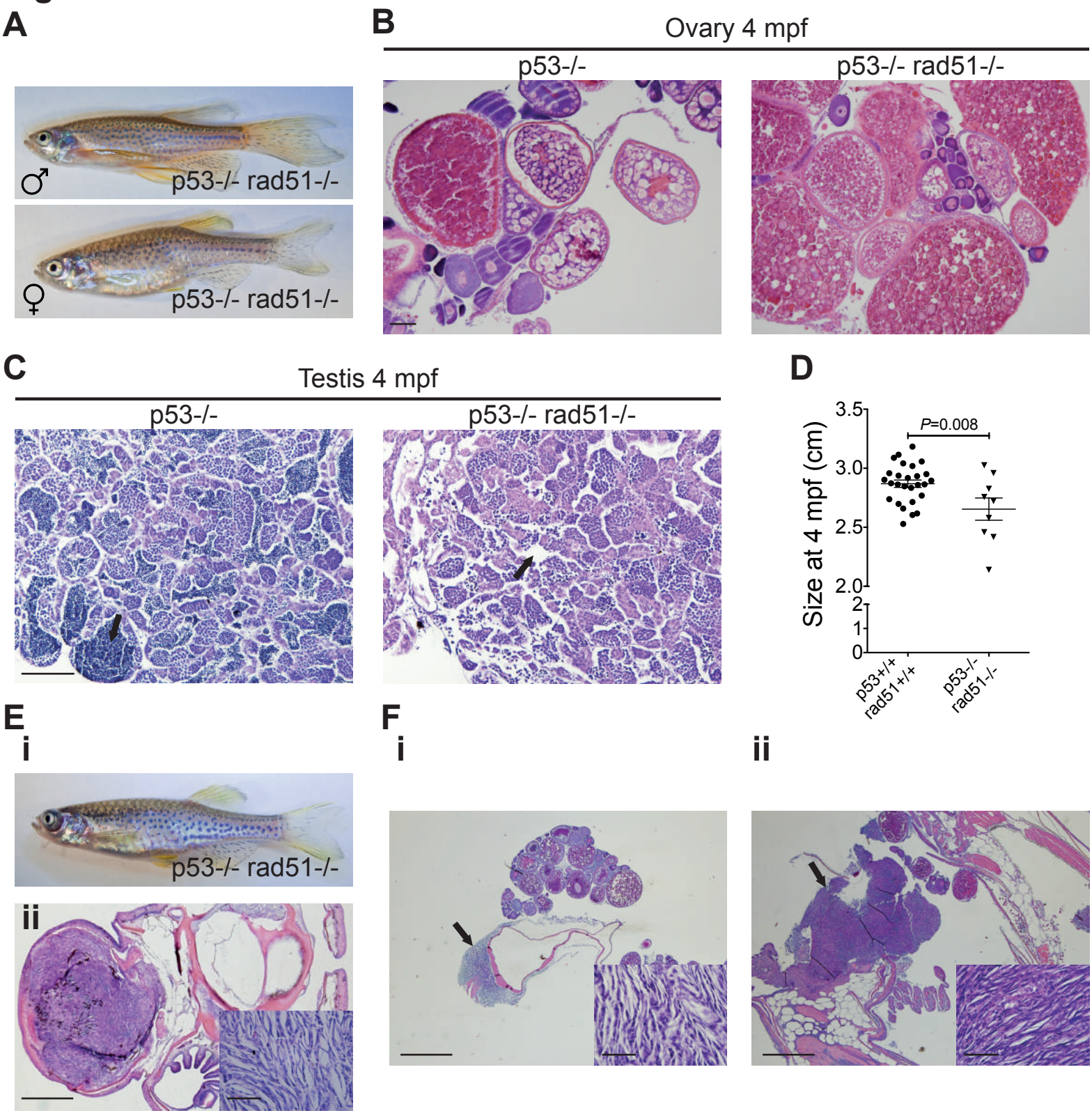

A
♂
p53-/- rad51-/-
♀
p53-/- rad51-/-
B
Ovary 4 mpf
p53-/-
p53-/- rad51-/-
C
Testis 4 mpf
p53-/-
p53-/- rad51-/-
D
Size at 4 mpf (cm)
3.5
3.0
2.5
2.0
2
1
0
P=0.008
p53+/+ rad51+/+
p53-/- rad51-/-
E
i
p53-/- rad51-/-
ii
F
i
ii

Figure S8

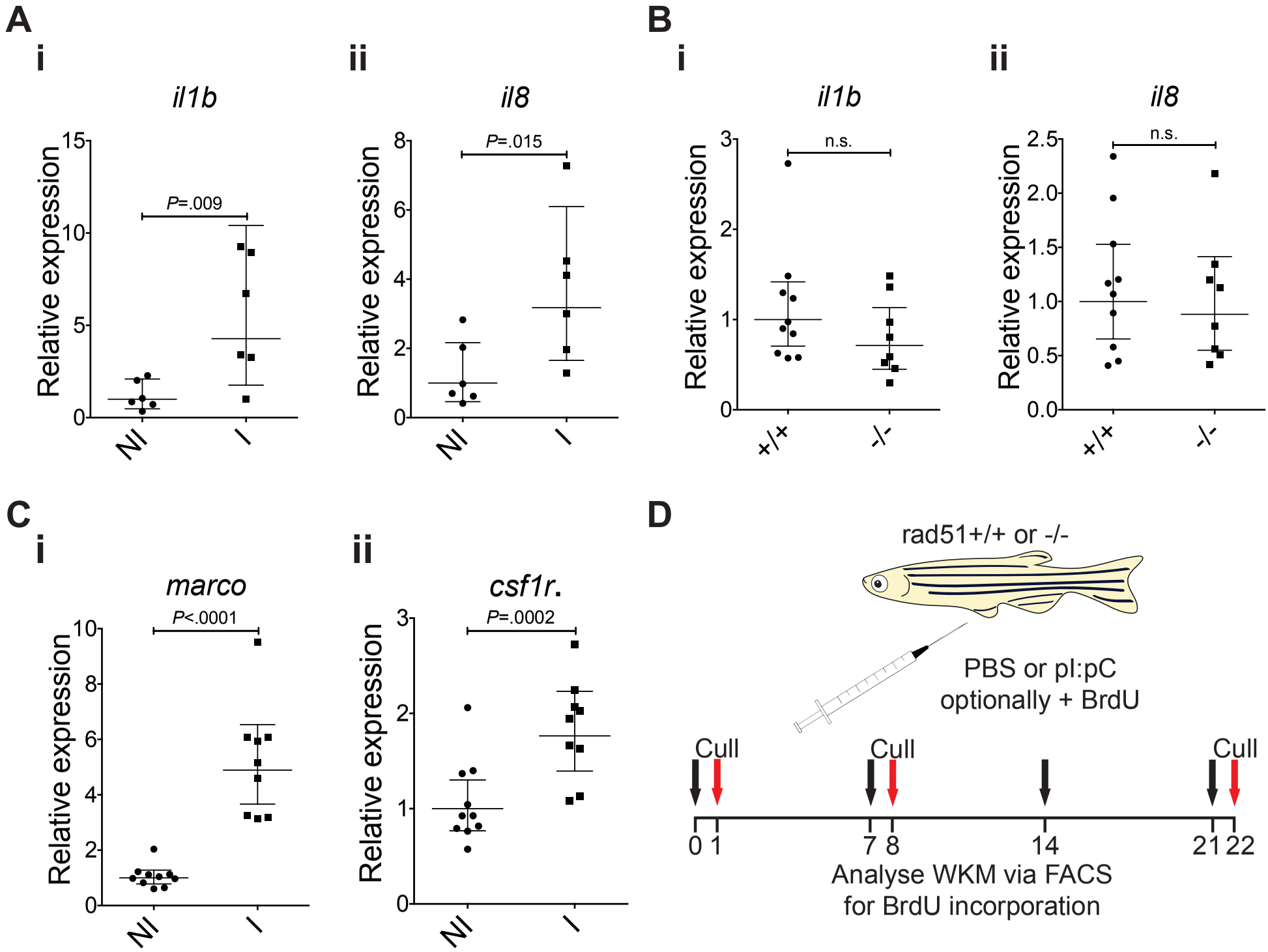

A
i
il1b
Relative expression
P=.009
NI
I
ii
il8
P=.015
B
i
il1b
n.s.
+/+
-/-
ii
il8
n.s.
C
i
marco
P<.0001
ii
csf1r.
P=.0002
D
rad51+/+ or -/-
PBS or pI:pC
optionally + BrdU
Cull
Cull
Cull
0 1
7 8
14
21 22
Analyse WKM via FACS
for BrdU incorporation

Figure S9

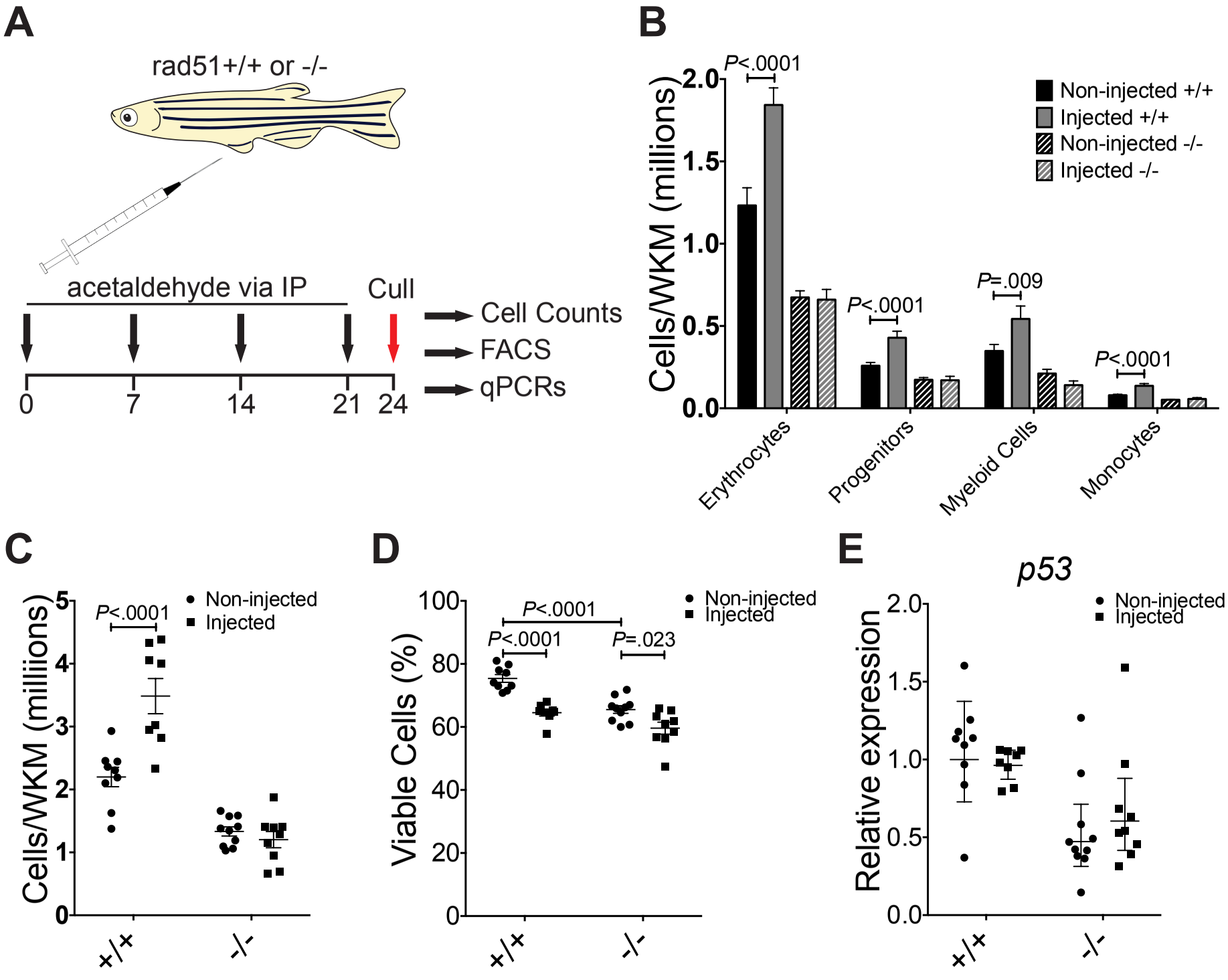

A
rad51+/+ or -/-
acetaldehyde via IP
Cull
0
7
14
21
24
Cell Counts
FACS
qPCRs
B
Cells/WKM (millions)
2.0
1.5
1.0
0.5
0.0
P<.0001
P<.0001
P=.009
P<.0001
Non-injected +/+
Injected +/+
Non-injected -/-
Injected -/-
Erythrocytes
Progenitors
Myeloid Cells
Monocytes
C
Cells/WKM (millions)
5
4
3
2
1
0
P<.0001
Non-injected
Injected
+/+
-/-
D
Viable Cells (%)
100
80
60
40
20
0
P<.0001
P<.0001
P=.023
Non-injected
Injected
+/+
-/-
E
p53
Relative expression
2.0
1.5
1.0
0.5
0.0
Non-injected
Injected
+/+
-/-

Table S1

Target	Species	Conjugate	Company	Catalog No	Dilution used
Rad51	rabbit	none	Abcam	ab137323	1:1000 (WB), 1:200 (IS)
Rad51	rabbit	none	Anaspec	55838	1:200
BrdU	rabbit	fluorescein	Roche	11202693001	1:100
pH2AX	rabbit	none	-	-	1:1000
Beta-actin	mouse	none	Sigma Aldrich	012M4821	1:1000
Mouse IgG	rabbit	HRP	Abcam	ab97046	1:50000
Rabbit IgG	goat	HRP	Molecular Probes	G21234	1:50000

Table S2

Target	Use	Forward	Reverse	Reference
il1b	qPCR	GCTGGAGATCCAAACGGATA	ATACGCGGTGCTGATAAACC	[1]
il8	qPCR	GTCGCTGCATTGAAACAGAA	AGGGGTCCAGACAGATCTCC	[1]
csf1r	qPCR	ATGACCATACCCAACTTTCC	AGTTTGTTGGTCTGGATGTG	[2]
marco	qPCR	ACGACAGCTTCGATAATTTG	AAAATACTGCTCTCGGTTCC	[2]
p53	qPCR	CGAGCCACTGCCATCTATAAG	TGCCCTCCACTCTTATCAAATG	
p21	qPCR	GTGTCAGGAAAAGCAGCAGA	GACGCTTCTTGGCTTGGTAG	

Table S3

Genotype	Staining intensity: High	Medium	Low
rad51+/+, p53+/+	5	9	3
rad51+/+, p53+/-	13	13	5
rad51+/+, p53-/-	5	4	6
rad51+/-, p53+/+	6	8	12
rad51+/-, p53+/-	25	17	17
rad51+/-, p53-/-	15	14	7
rad51-/-, p53+/+	1	6	6
rad51-/-, p53+/-	4	6	19
rad51-/-, p53-/-	6	3	2

Unsuccessful genotyping	3
Successful genotyping	237

Pooled data from 3 separate clutches with 60 embryos each