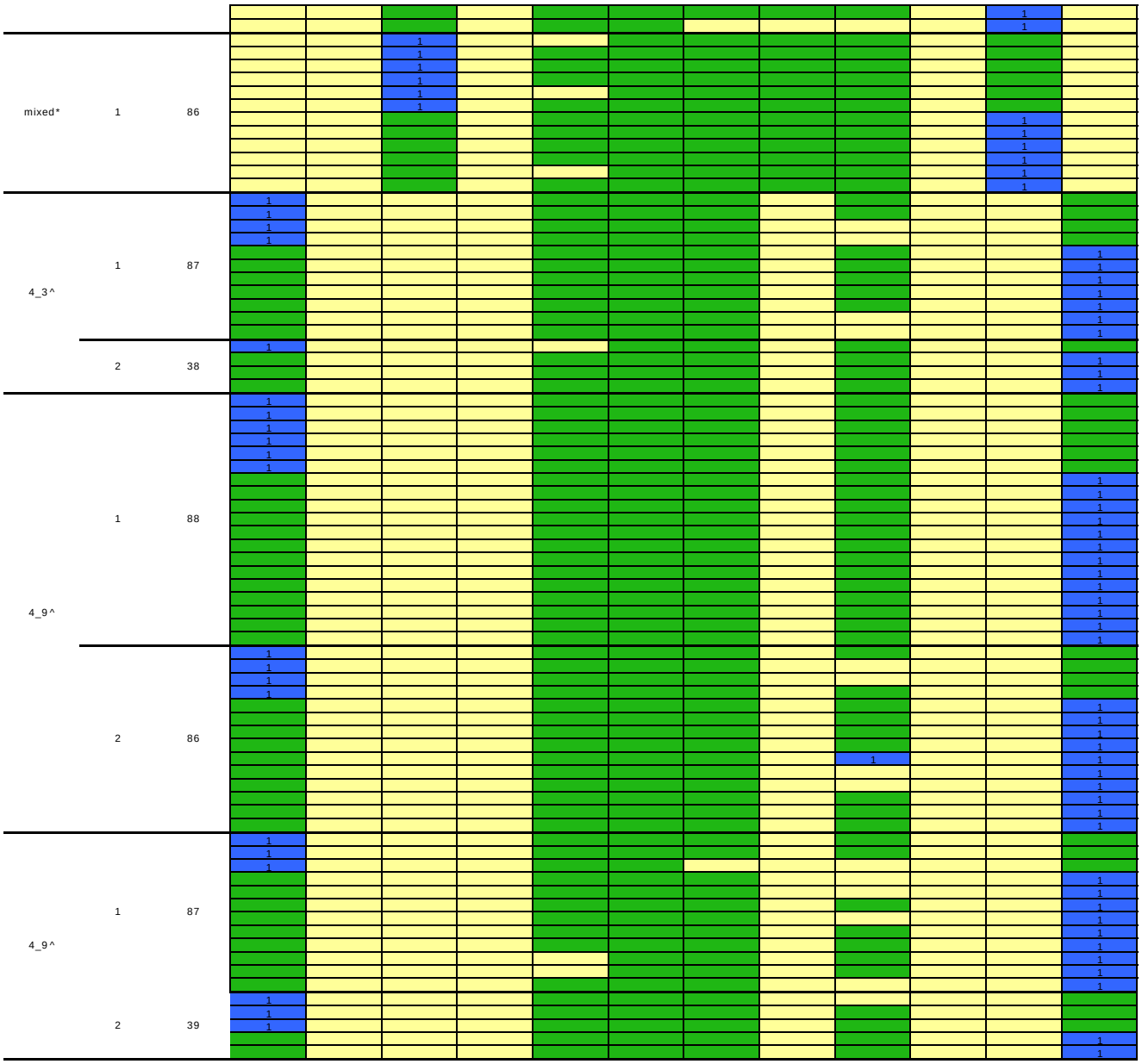
Table S1. Sequence of primers used for positional cloning analysis

Name	SSLP/SNP	Forward primer (5'-3')	Reverse primers (5'-3')
z11989	SSLP	ATGTGTCGACGGTGTGAAAA	GCAAGAGTGTGTGCGTGC
176	SSLP	GCACTGGCTGGTAGTGACCT	AAAAGCGCTTAAGATGCAACA
227	SSLP	GATTTTTCACTTGAGGGAGCA	AAAAACACTGGCATCATTTGC
245	SSLP	GGAAATCAGCACTATTATGACCA	CGTCGTAGAAAAGTTGTTGTATGC
z3816	SSLP	AGATCAGGTGCACAGTGGCG	CCACTGAAGGGCAAGTGGCT
cpsf1 3' UTR	SNP	TGACAGAATCACGCATTAACTTTT	CAACATGAACGACATGGTCA
cpsf1 ex 9	SNP	AGAGCCAAAATTTGAATGTCAG	TGCAAAATATCTCACATTTCTCC
zH49K18T7	SNP	CCATCTTGAGATGCAGCAGA	GCAAATGCCTCCTTTACACC
spag1 ex 6	SNP	GGAGCCATTCAGTCAGCAAT	GGGAGCGTTATTGGCTTGT
rnf19 ex 7	SNP	TTTCCATGATGCTTCAAACCT	TTCAAAATAACCTCCCCAACA

SUPPLEMENTAL TABLE S2

Family	Plate n. (96 well)	n. of mutants (each plate)	Name of Marker (Kind) 204 (SSLP)	z11989 (SSLP)	176 (SSLP)	227 (SSLP)	CPSF1 3' UTR (SNP)	CPSF1 EX9 (SNP)	zH49K18T7 (SNP)	SPAG1ex6 (SNP)	RNF19ex7 (SNP)	245 (SSLP)	z3816 (SSLP)	148 (SSLP)
3_3b*	1	87			1									
					1									
													1	
													1	
													1	
													1	
3_5*	1	47			1	1	1							
													1	
													1	
													1	
													1	
													1	
													1	
	2	88											1	
													1	
													1	
													1	
													1	
	3	31								1	1	1	1	
3_6c^	1	88		1										
				1										
				1	1									
				1										
				1										
				1										
				1										
				1										
				1										
				1										
													1	
													1	
													1	
	2	86		1										
				1	1									
				1										
				1										
				1										
				1										
				1										
				1										
				1	1									
													1	
													1	
													1	
													1	
													1	
	3	78			1									
					1									
					1									
													1	
													1	
													1	
													1	
3_15*	1	65			1									
					1									
													1	
													1	
													1	
													1	
3_6c_m x 3_15_f^	1	88			1									
					1									
					1									
					1									
					1									
													1	
													1	
													1	
													1	
													1	
													1	
	2	88			1									
					1									
													1	
													1	
													1	
													1	
													1	
													1	
	3	88			1									
					1									
					1									
					1									
													1	
													1	
													1	
													1	
	4	88			1									
					1									
													1	
													1	
													1	
													1	
													1	
	5	87			1									
					1									
					1									
					1									
					1									
													1	
													1	
													1	
													1	
													1	
													1	
	6	70			1									
					1									
					1									
													1	



recombinants			21	17	37	1	1	0	0	1	2	2	70	44
total mutants		1590	425	174	1165	166	1566	1590	1588	1163	1574	166	1165	425
cM from mutation			3.29411765	6.51340996	2.11731044	0.40160643	0.04257131	0	0	0.05732302	0.08470987	0.80321285	4.00572246	6.90196078

LEGEND

	MUT genotype
	Not performed or failed
	HET genotype (recombinant)
Family:	Pair of incrossed HETs
Mutant:	Phenotypically mutant 5 dpf embryos
*	Scored by reduced mpx by WISH at 5 dpf
^	Scored by abnormal morphology at 5 dpf
SSLP	Single Strand Length Polymorphism
SNP	Single Nucleotide Polymorphism
cM	Centimorgans (n. of recombinants/n. of mutants)*100/1.5

Table S3. Sequence of *cpsf1* morpholino used in the project

Name	Sequence (5'-3')
drCPSF1 -28ATG MO	GAAGATCAGCGGCTCCTCTCAGCAC
drCPSF1 -3ATG MO	CCTGACGGTACACCGCGTACATGAT
drCPSF1 ATG MO	GAGCCTGACGGTACACCGCGTACAT
drCPSF1 +3ATG MO	GGTGAGCCTGACGGTACACCGCGTA
drCPSF1 e9i9 MO	ATGCAAAATATCTCACATTTCTCCT
drCPSF1 mismatch MO	TGAcCCTcACGcTACACgGCGTgAT

Table S4. S equence of primers used for RT-PCRs, qRT-PCRs, WISH probe cloning, and poly(A) length assays

Name	Forward primer (5'-3')	Reverse primers (5'-3')
*cpsf1*moprhants RT-PCR	CTGGTGGTTCTGCCTTTCAG	GAGCCCAAAAACAGATAGCC
cpsf1 WISH probe	ACTTCTAGCGCGAGTCGTTC	GGGCTCGAACAGAATAAGCA
cpsf1 qPCR	CAACTTCATCTCCAGCCAAGA	CCACCTGCTCCAGTTTCTCT
ef1a qPCR	TTGAGAAGAAAATCGGTGGTGCTG	GGAACGGTGTGATTGAGGGAAATTC
Snrnp70 poly(A) length assay	AACGTTTCATGGGTTAGTACATTT	CAAGAATTTAAAGCAGAACAAATACG

Figure S1. Additional WISH stainings

(A-B) WISH for *l-plastin*: lateral view, anterior to the left, dorsal upwards. *l-plastin* expression is lost in 5 dpf *grechetto* mutants (B).

(C-D) WISH for *lysozymeC*: lateral view, anterior to the left, dorsal upwards. *lysozymeC* expression is lost in 5 dpf *grechetto* mutants (D).

(E-F) WISH for *gata1*: lateral view, anterior to the left, dorsal upwards. *gata1* expression is lost in 5 dpf *grechetto* mutants (F).

(G-H) WISH for *rag1*: ventral view, anterior to the left. *rag1* expression is lost in 5 dpf *grechetto* mutants (H).

(I-J) WISH for *gata6*: lateral view, anterior to the left, doral upwards. *gata6* expression is normal in the heart of 5 dpf *grechetto* mutants (J, arrow) but lost in the gut (J, asterisk) compared to WT siblings (I).

(K-L) WISH for *pdx1*: ventral view, anterior to the left. *pdx1* expression is normal in the pancreas of 5 dpf *grechetto* mutants (L, arrow) but lost in the gut (L, asterisk) compared to WT siblings (K).

Figure S2. Validation of cpsf1 as the gene responsible for the grechetto phenotype

(A-E) A compound *cpsf1*^{zdfl8a12}/*cpsf1*^{hi2675} heterozygote embryo shows typical features of a *grechetto* mutant in lateral (A) and dorsal (B) brightfield view, including a markedly decreased number of melanophores in the lateral stripe (B, quantified in D) and decreased iridophores on epi-illumination of the dorsal region (C, quantified in E).

(F) Morpholino-mediated knock-down strategy for *cpsf1*. A splice morpholino targeting exon 9 splice donor site was designed along with a non-targeting morpholino as control.

Left, primers designed on exons 5 and 10 were used to morphotype embryos at 24 hpf. Middle, RT-PCR showed that injection of *cpsf1* e9i9 morpholino but not of control morpholino resulted in an abnormally spliced RT-PCR product, whose amount increased with the dose of the morpholino injected. Right, sequencing of the RT-PCR demonstrated that the abnormally spliced mRNA retains intron 9 in its coding sequence, leading to a premature stop codon.

(G-H) WISH for *mpx* in $p53^{m/m}$ embryos: lateral view, anterior to the left, dorsal upwards. *mpx* expression is lost in 5 dpf *cpsf1* morphants (H) but not in control morpholino injected embryos (G).

(I-J) Ventral view of alcyan blue staining of the jaw cartilages in $p53^{m/m}$ embryos. 5 dpf *cpsf1* morphants show abnormal development of the posterior ceratohyal and ceratobranchial cartilages (J) compared to control morpholino injected embryos (I).

(K-P) 5 dpf $p53^{m/m}$ embryos injected with a *cpsf1* morpholino show typical features of a *grechetto* mutant in dorsal (N) and lateral (O) brightfield view, including a markedly decreased number of iridophores on epi-illumination of the dorsal region (P) compared to control morpholino injected embryos which appeared normal (K-M).

Figure S3. Neural crest specification, migration, and differentiation, and jaw development in *grechetto* mutants

(A-J) WISH for *sox10* (A-B), *foxD3* (C-D), *crestin* (E-F), *dlx2* (G-H), *pax9a* (I-J) in WT siblings (A, C, E, G, I) and *grechetto* mutants (B, D, F, H, J) at the indicated timepoints. (A-J), lateral view, anterior to the left, dorsal upwards. (Ai-Ji), dorsal view, anterior to the top. *Sox10* (B, arrow) and *dlx2* (H, arrow) expression in *grechetto* mutants is slightly reduced compared to WT siblings (A, G, arrows).

(K-P) Acridine orange staining in WT siblings (K, M, O) and *grechetto* mutants (L, N, P) at the indicated timepoints. Ventral view, anterior to the left. *Grechetto* mutants (L-N-P) show increased cell death in the jaw region compared to WT siblings (K-M-O).

Figure S4. Activatted Caspase-3 staining of *grechetto* mutants and WT siblings

(A-D) Single slice confocal microscope images of anti-activated caspase3 whole mount immunostaining of 4 dpf *grechetto* mutants (C-D) and WT siblings (A-B) in ventral (A, C) and lateral (B, D) views. Panels result from merging the red and brightfield channels. Mutants showed the presence of apoptotic cells in ventral view of the jaw region that were not found in WT siblings (C, arrow), and apoptosis in the jaw, brain and eyes (D, arrow, star and arrowhead, respectively) in lateral view.

(E-H) Epifluorescence images of anti-activated caspase3 whole mount immunostaining of 4 dpf *grechetto* mutants in lateral (E-F),ventral (E-F, insets) and dorsal (G-H) views. *Grechetto* mutants show increased apoptotic cell death in the jaw, brain, eye and gut (F, H, arrows) regions compared to WT siblings (E, G).

Figure S5. Loss of *cd41:EGFP^{low}* cells in *grechetto* mutants

(A-F). Live imaging of the body region of *cpsf1^{zdfl8a12}*;Tg(*cd41*:EGFP) embryos at 72 hpf (A-B), 96 hpf (C-D), 120 hpf (E-F) *grechetto* mutants (B, D, F) and WT siblings (A, C, E). Lateral view, anterior to the left, dorsal upwards. In *grechetto* mutants, EGFP^{low}+ cells are decreased in number at 72 hpf (B) and 96 hpf (D) and are almost undetectable at 120 hpf (F) compared to WT siblings (A, C, E).

(G) quantification of the above experiments. EGFP^{low}+ cells as counted in the CHT of 15 WT and mutant embryos. The number of cells in mutants is plotted as ratio to the number of

cells in WT siblings at the same stage, normalized to 1. **$P \leq .005$; ***$P \leq .0005$ (Student *t* test).

Figure S6. HSC TUNEL staining of *grechetto* mutants and WT siblings

(A-H) TUNEL staining of 3.5 dpf Tg(*c-myb*:EGFP);*cpsf1*^{zdfl8a12} WT (A-D) and mutant (D-H) embryos, in transverse sections taken at the distal end of the yolk extension. (A, E) green indicates cells expressing EGFP under the control of the *c-myb* promoter. (B, F) red indicates TUNEL-positive cells. (C, G) merge of the green and red channels show a partial overlap between the TUNEL signal and EGFP expression. (D, H) merge of the red, green, DAPI signals. Insets indicate the anatomical location of the cropped region (between the notochord and the pronephric duct region).

FIGURE S1

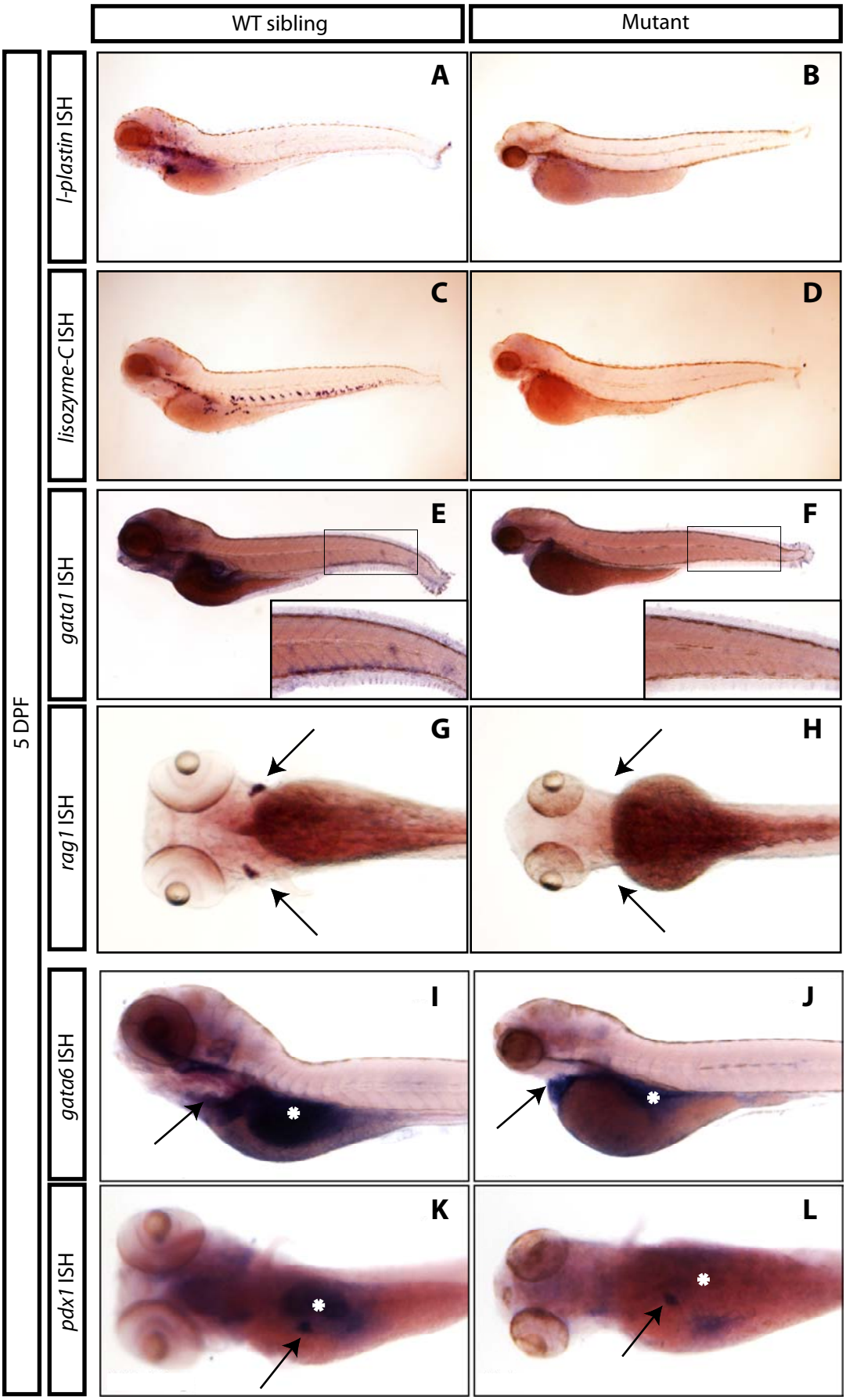

WT sibling
Mutant
5 DPF
l-plastin ISH
lisozyme-C ISH
gata1 ISH
rag1 ISH
gata6 ISH
pdx1 ISH
A
B
C
D
E
F
G
H
I
J
K
L

FIGURE S2

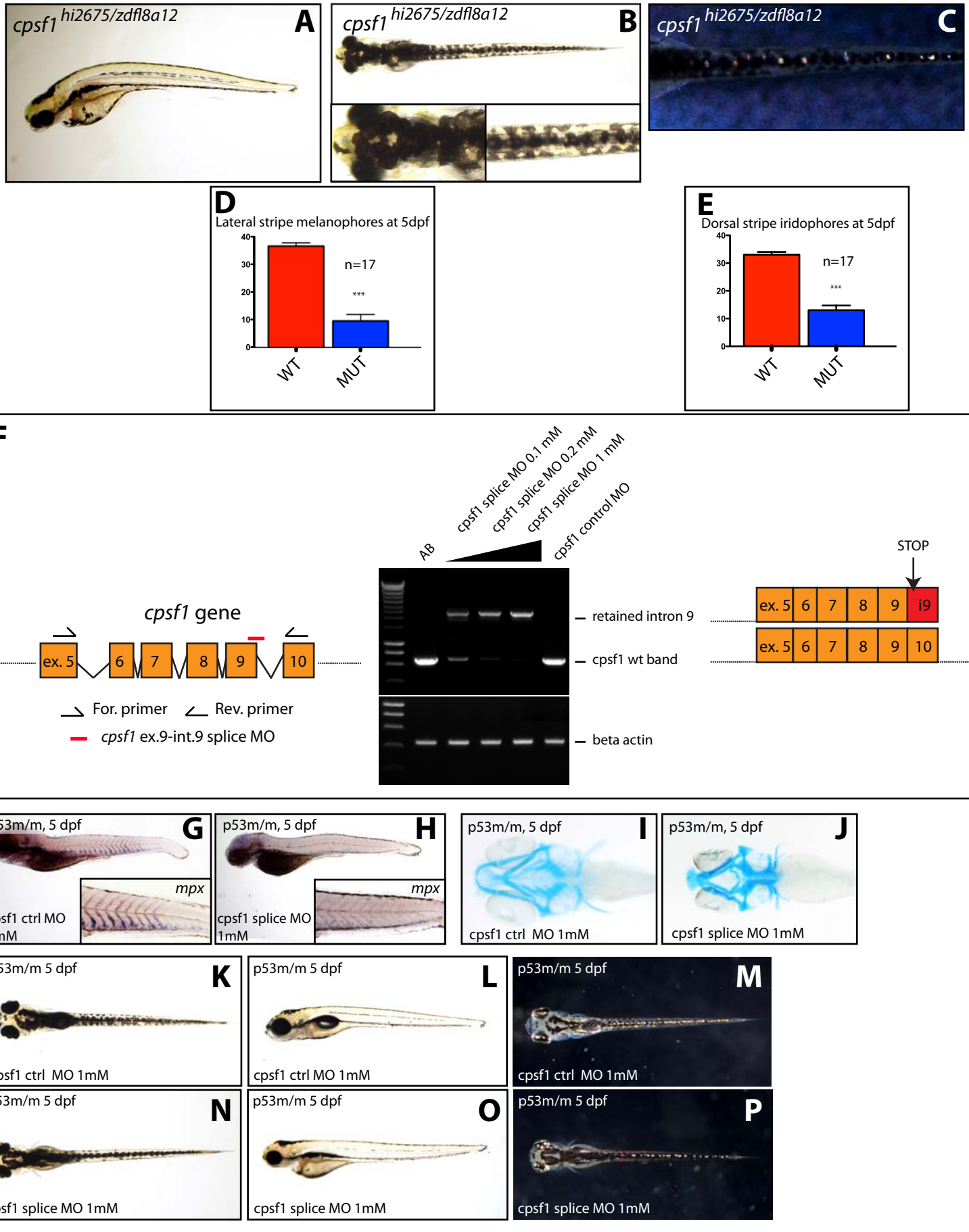

cpsf1 hi2675/zdfl8a12
A
cpsf1 hi2675/zdfl8a12
B
cpsf1 hi2675/zdfl8a12
C
D
Lateral stripe melanophores at 5dpf
n=17
***
WT
MUT
E
Dorsal stripe iridophores at 5dpf
n=17
***
WT
MUT
F
cpsf1 splice MO 0.1 mM
cpsf1 splice MO 0.2 mM
cpsf1 splice MO 1 mM
cpsf1 control MO
AB
cpsf1 gene
ex. 5
6
7
8
9
10
For. primer
Rev. primer
cpsf1 ex.9-int.9 splice MO
retained intron 9
cpsf1 wt band
beta actin
STOP
ex. 5
6
7
8
9
i9
ex. 5
6
7
8
9
10
p53m/m, 5 dpf
G
mpx
cpsf1 ctrl MO 1mM
p53m/m, 5 dpf
H
mpx
cpsf1 splice MO 1mM
p53m/m, 5 dpf
I
cpsf1 ctrl MO 1mM
p53m/m, 5 dpf
J
cpsf1 splice MO 1mM
p53m/m 5 dpf
K
cpsf1 ctrl MO 1mM
p53m/m 5 dpf
L
cpsf1 ctrl MO 1mM
p53m/m 5 dpf
M
cpsf1 ctrl MO 1mM
p53m/m 5 dpf
N
cpsf1 splice MO 1mM
p53m/m 5 dpf
O
cpsf1 splice MO 1mM
p53m/m 5 dpf
P
cpsf1 splice MO 1mM

FIGURE S3

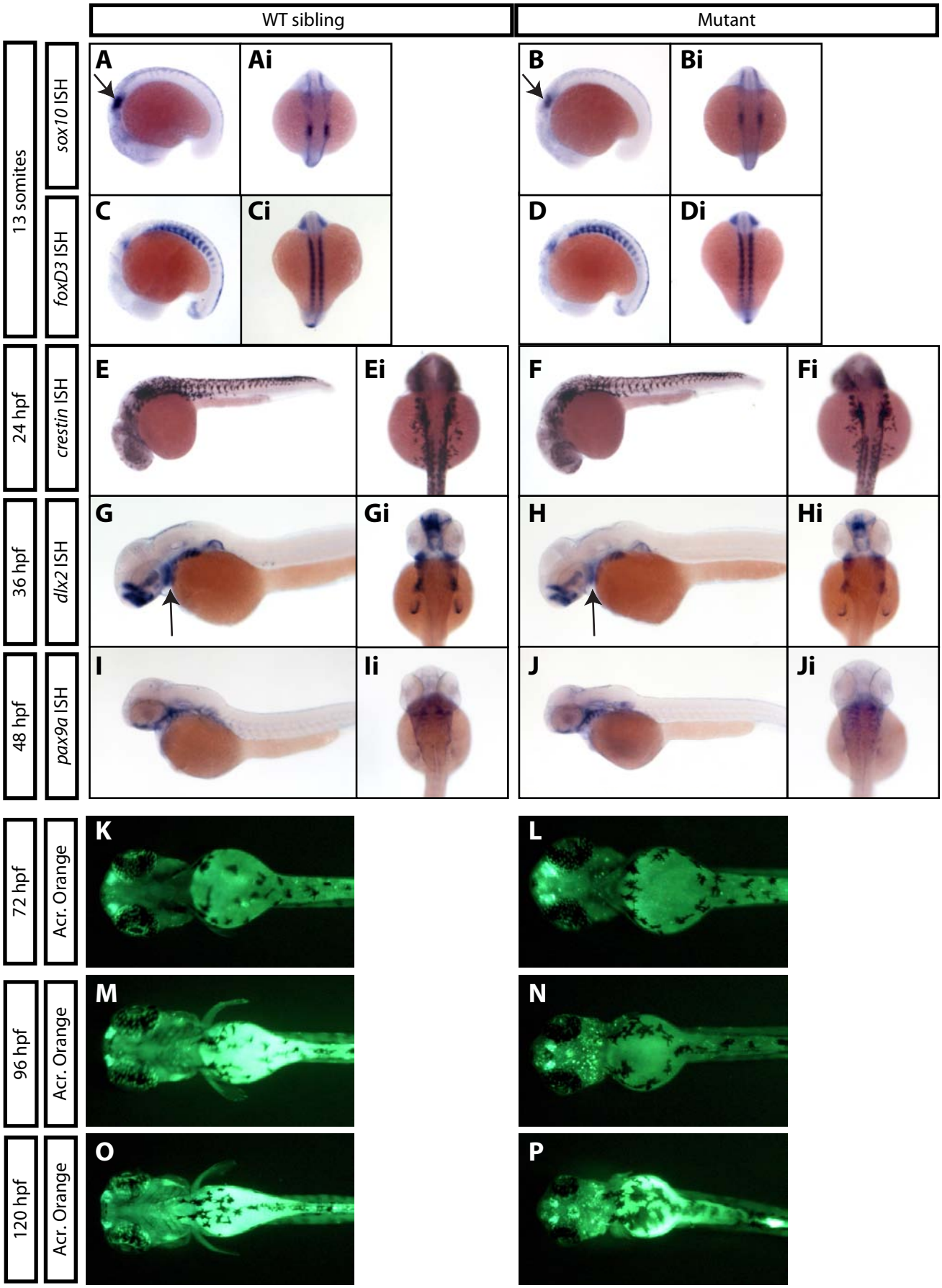

WT sibling
Mutant
13 somites
sox10 ISH
foxD3 ISH
24 hpf
crestin ISH
36 hpf
dlx2 ISH
48 hpf
pax9a ISH
72 hpf
Acr. Orange
96 hpf
Acr. Orange
120 hpf
Acr. Orange
A
Ai
B
Bi
C
Ci
D
Di
E
Ei
F
Fi
G
Gi
H
Hi
I
Ii
J
Ji
K
L
M
N
O
P

FIGURE S4

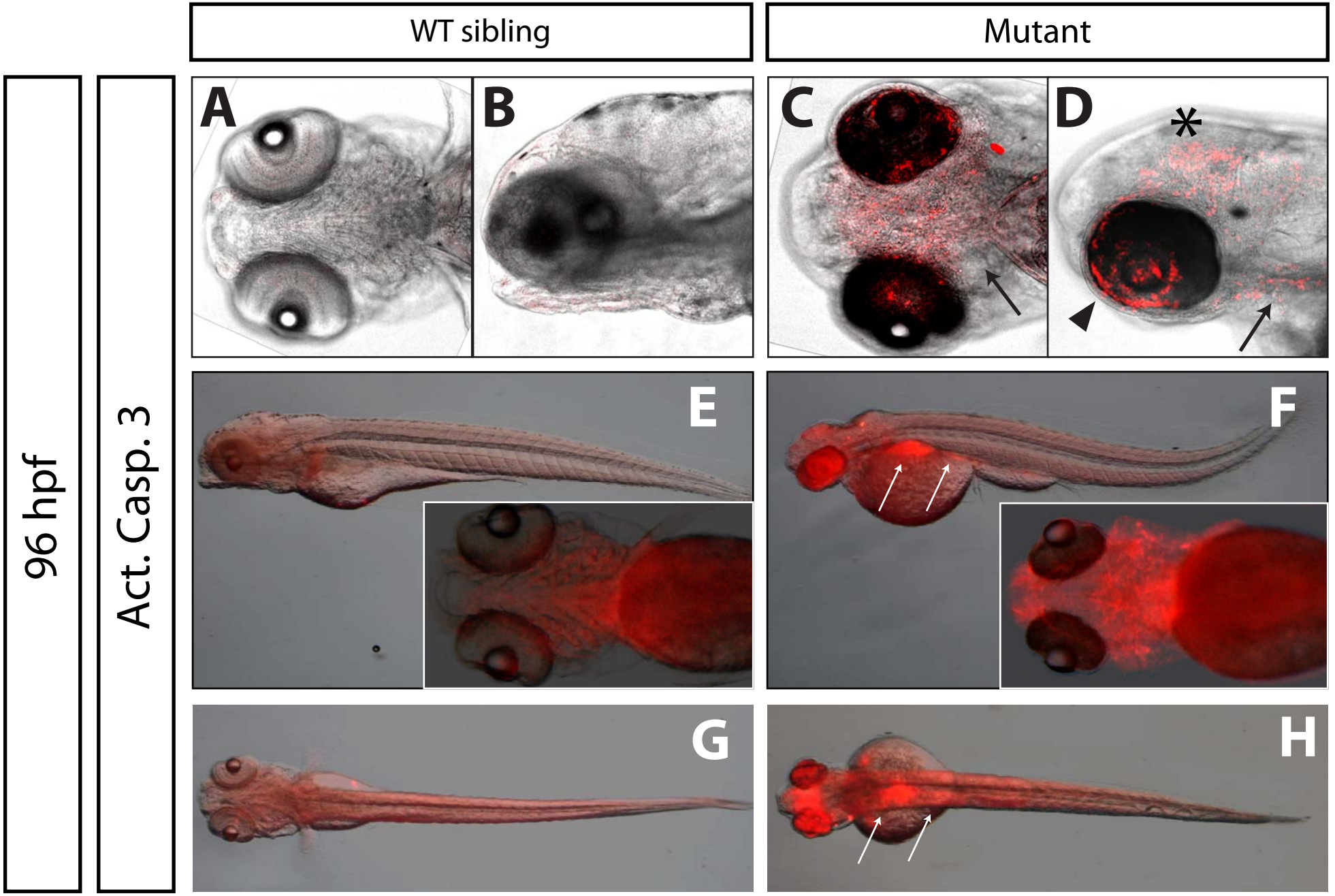


FIGURE S5

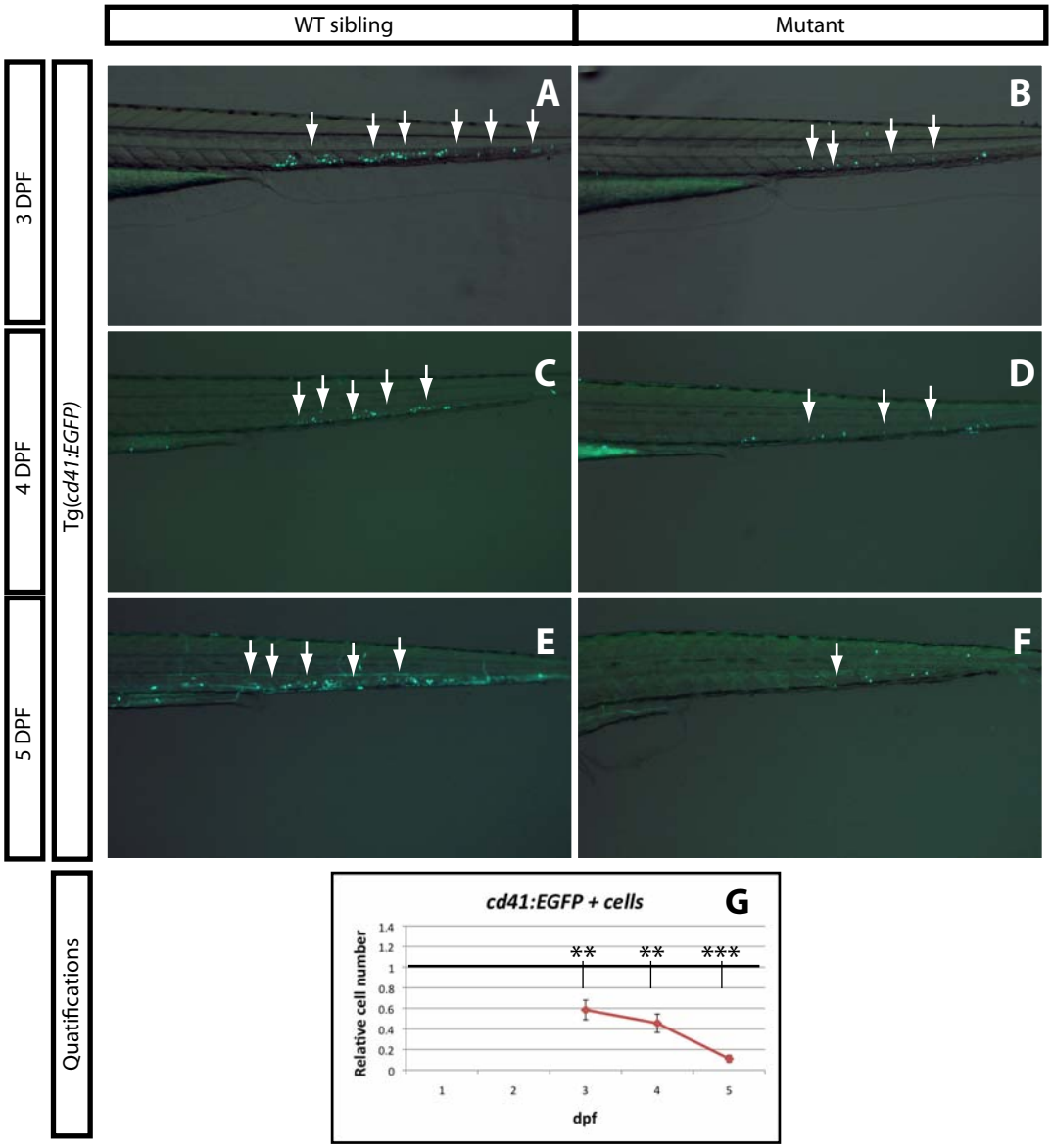

WT sibling
Mutant
3 DPF
4 DPF
5 DPF
Tg(cd41:EGFP)
A
B
C
D
E
F
Quatifications
cd41:EGFP + cells
G
Relative cell number
dpf
**
**
***

FIGURE S6