

Anatomy and molecular composition of the spinal lesion site in zebrafish

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1. Abstract

Background: Unlike mammals, zebrafish can successfully regenerate their spinal cord after sustaining a lesion. This interspecies difference in regenerative capacity is partly attributed to differences in the anatomy and molecular composition of the lesion site, rendering a more permissive environment in zebrafish, compared to that of mammals. A key determinant of the environment's conductivity to axonal regeneration is the composition of the extracellular matrix (ECM), modulated by fibroblasts, astrocytes and cells of the immune system. Growing evidence supports that the immune system is a critical modulator of the quality of regenerative outcome in many regeneration systems. Previous studies have shown that in the absence of macrophages, axonal regeneration is strongly impaired, however, the exact role of macrophages in this process is not clear.

Hypothesis: Macrophages invading the lesion site promote axon regeneration by modulating ECM deposition in the spinal cord lesion site of zebrafish.

Methods: We used *in situ* hybridization to investigate mRNA expression of 13 genes coding for ECM components, previously shown to be specifically upregulated in the spinal lesion site of zebrafish in response to lesioning, in wild-type (*wik*) and *irf8*^{-/-} zebrafish larvae (do not develop mature and functional macrophages or microglia until after 31 dpf), after spinal cord injury.

Results: We found no difference in expression levels of the selected genes between *wik* and *irf8*^{-/-} zebrafish larvae, thus we conclude that macrophages do not regulate these genes at the transcriptional level.

2. Methods

Experimental Design

Zebrafish larvae lesioned 3 days post fertilization

Image analysis

Zebrafish larvae fixed with 4% PFA 1 day post lesion

Larvae visualised and pictured

Standard *in situ* hybridization protocol targeting selected genes

Larvae mounted on microscope slides

Stab lesions were performed using a 30.5 G needle to sever the spinal cord at the dorsal trunk of the larvae, at height of the anal pore.

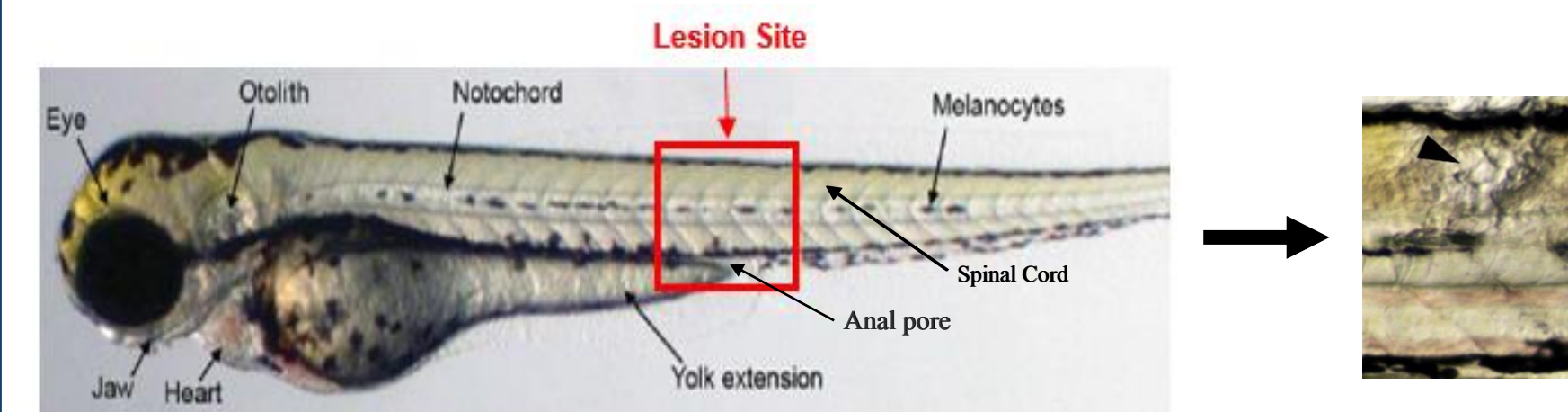


Figure 1:

Image (left) showing an unlesioned 3 dpf zebrafish larvae (*wik*) with its major features and lesion site labelled.

Image (right) showing the lesion site after a stab lesion

3. Results

	Fn1a		Fn1b		Col 1a1a		Col 1a1b		Col 1a2		Col 5a1	
	WIK	IRF8	WIK	IRF8	WIK	IRF8	WIK	IRF8	WIK	IRF8	WIK	IRF8
++	13	13	5	7	6	9	13	12	21	18	—	—
+	—	—	—	—	—	2	—	—	—	3	9	10
-	—	—	—	—	—	—	—	—	—	—	—	—
n	13	13	5	7	6	11	13	12	21	22	9	10

	Col 5a2a		Col 5a3b		Col 6a2		Col 12a1a		Col 12a1b		Col 27a1a		MMP9	
	WIK	IRF8	WIK	IRF8	WIK	IRF8	WIK	IRF8	WIK	IRF8	WIK	IRF8	WIK	IRF8
++	—	—	8	12	—	—	14	16	12	12	11	15	10	11
+	9	—	2	2	—	—	—	—	1	—	—	—	6	2
-	—	12	—	—	10	9	—	—	—	—	—	—	—	—
n	9	12	10	14	10	9	14	16	13	12	11	15	16	13

Tables A + B

Tables showing the scoring of staining for each gene analysed.

++ = Strong Staining, + = Weak Staining, - = No staining, n = Total number of zebrafish embryos

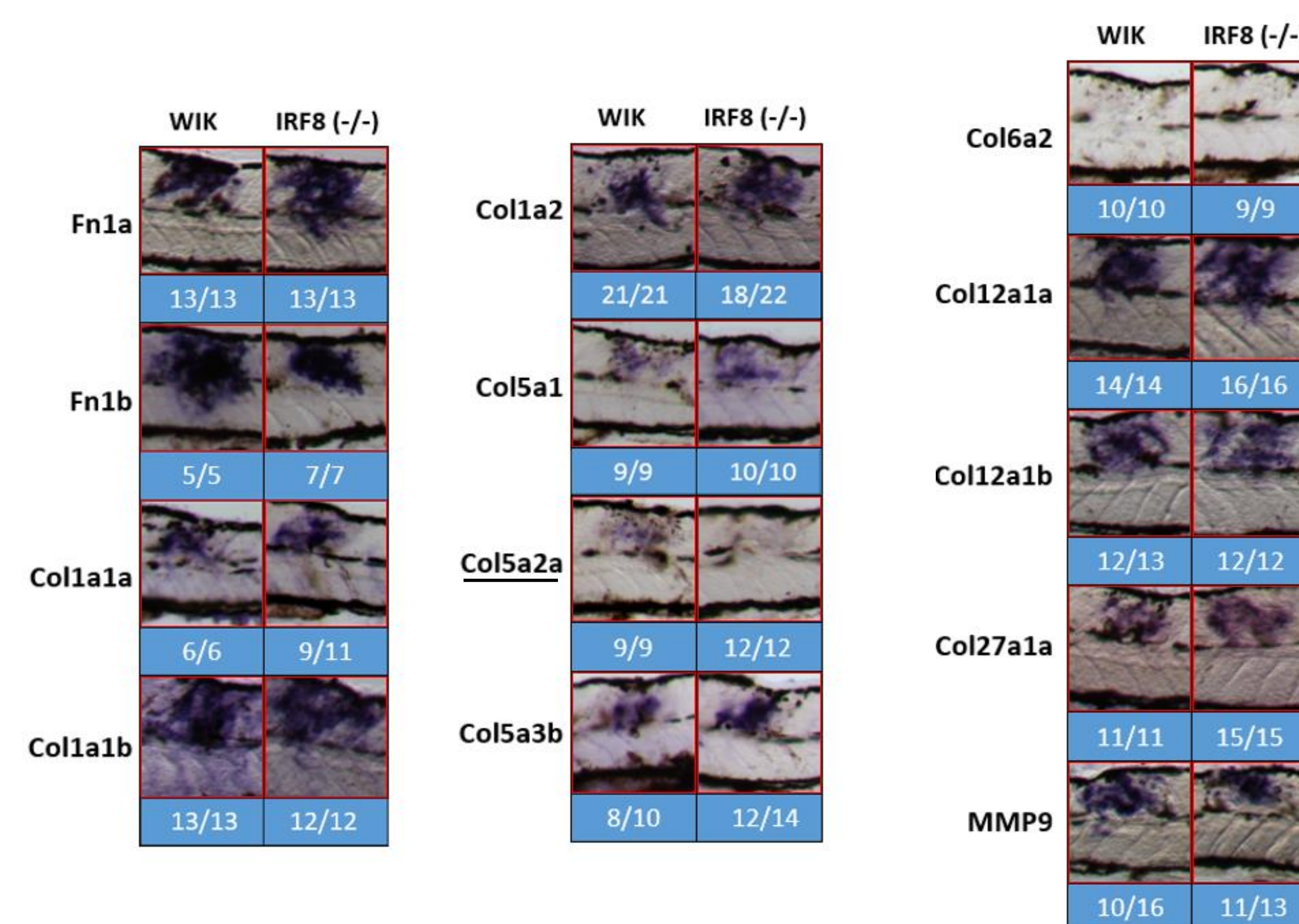


Figure 2:

Representative images of average staining in the lesion site for each gene in 4 dpf *wik* and *irf8*^{-/-} zebrafish larvae, 1 dpl.

Lateral view of the larvae is shown (rostral is left, dorsal is up)

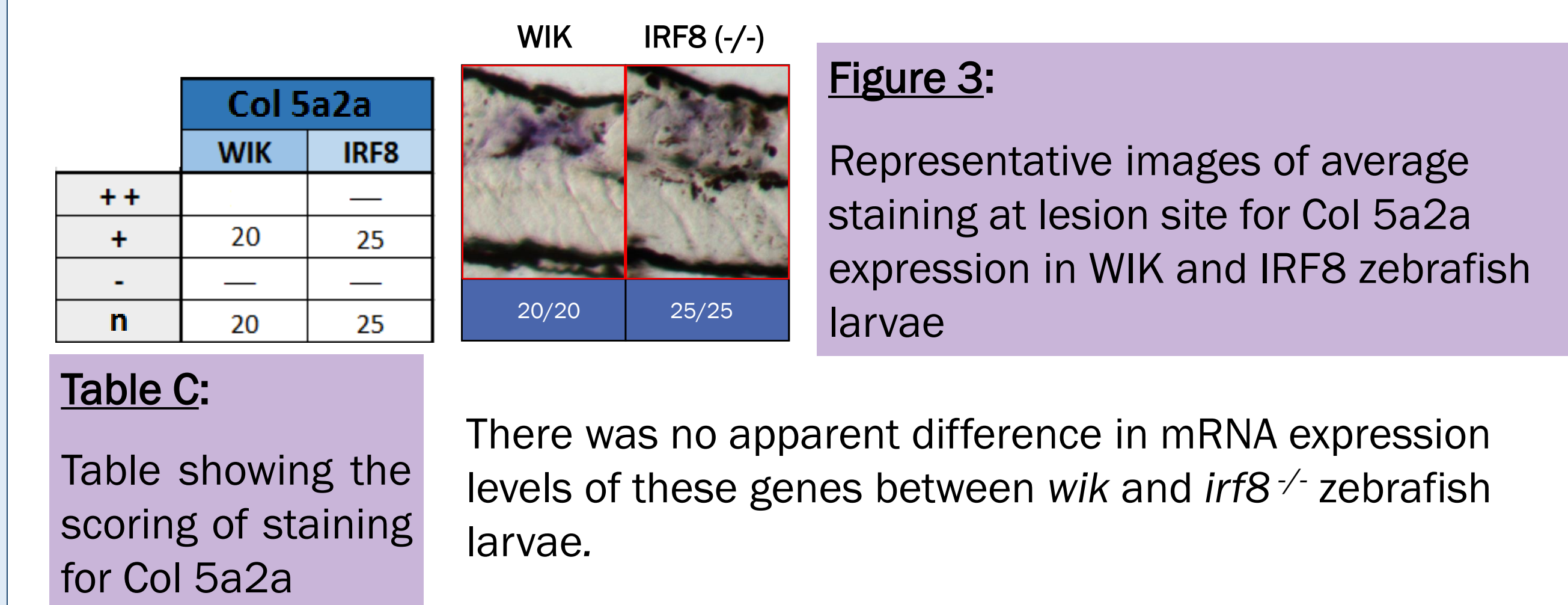


Figure 3:

Representative images of average staining at lesion site for Col 5a2a expression in WIK and IRF8 zebrafish larvae

Table C:

Table showing the scoring of staining for Col 5a2a

There was no apparent difference in mRNA expression levels of these genes between *wik* and *irf8*^{-/-} zebrafish larvae.

4. Conclusion

- The absence of macrophages did not appear to affect the upregulation of expression of these genes observed at the lesion site in response to spinal cord injury.
- We conclude that macrophages do not regulate these genes at the transcriptional level.
- This does not rule out modulation of these genes by macrophages downstream of transcription.
- These experiments must be repeated to confirm the current findings

5. Future Experiments

Short Term

Immunohistochemistry – protein level

Long Term

Cytokine expression levels

Expression profiling to identify more genes that are differentially regulated upon loss of macrophages (WT) and injury

6. Acknowledgements

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