

9.3 USB files

9.3.1 Movies

Movie 9.1. Evans blue labelling co-localised with a second harmonic signal from myofibers throughout the extent of the injury field.

7dpf *pax7a:pax7a:eGFP* larvae were submerged in 0.1% Evans blue for 1h, injured and then imaged on a multiphoton microscope. Visualisation of Evans blue in damaged myofibers (magenta), second harmonic generation (SHG) in myofibers (cyan) and *pax7a:eGFP* (muSC; green) reveals extensive co-localisation of the Evans Blue and SHG signal.

Scale bar: 100µm.

Movie 9.2. muSC response to single myotome injury.

Injured 3 dpf *pax7a:eGFP*⁺ larvae were imaged by multiphoton microscopy from 1-16.83 hpi with 10 minute intervals between frames. Acquisition encompassed the entire injured myotome and z-stack projections, reveals the migration of muSCs in response to injury.

Scale bar: 50µm. Time stamp in minutes post injury.

Movie 9.3. *Tp1:H2BmCherry*; *Tp1:VenusPEST*-expressing cells respond to muscle injury.

5 dpf *Tp1:H2BmCherry*; *Tp1:VenusPEST*⁺ larvae were injured and live imaged on a multi-photon system from 1-18.75 hpi with 15 minute intervals between frames. Live imaging reveals a population of migratory mCherry⁺Venus⁺ cells responding towards muscle injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.4. Activated muSCs do not express *Tp1:H2BmCherry* in response to injury.

7 dpf *pax7a:eGFP*; *Tp1:H2BmCherry*⁻ expressing larvae were injured and live imaged on a multi-photon system from 1-10.2 hpi with 25 minute intervals between frames. Live imaging reveals that proliferating and migrating *pax7a:eGFP*-expressing muSCs did not express the *Tp1:H2BmCherry* transgene following injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.5. *pax7a:eGFP*-expressing muSCs express *Tp1:VenusPEST* in response to injury.

5 dpf *pax7a:eGFP*; *Tp1:VenusPEST*⁻ expressing larvae were injured and live imaged on a multi-photon system from 1.5-18.3 hpi with 21 minute intervals between frames. Live imaging reveals proliferating and migrating eGFP⁺Venus⁺ in response to injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.6. Proliferating *pax7a:eGFP*-expressing muSCs express *Tp1:VenusPEST* in response to injury.

5 dpf *pax7a:eGFP*; *Tp1:VenusPEST*⁻ expressing larvae were injured and live imaged on a multi-photon system from 1.5-18.3 hpi with 21 minute intervals between frames. Live imaging highlights that proliferating *pax7a:eGFP*-expressing muSCs and the subsequent daughter cells expressed *Tp1:VenusPEST* in response to injury.

Scale bar: 10 µm. time stamp in minutes post injury.

Movie 9.7. Neutrophils and macrophages are recruited towards muscle injury.

5 dpf *mpx:GFP*; *fms:mCherry*⁺ larvae were injured and treated with DMSO and live imaged on a multi-photon system from 0.5-3.4 hpi with 8.6 minute intervals between frames. Live imaging demonstrates recruitment of GFP⁺ neutrophils and mCherry⁺ macrophages towards muscle injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.8. Fewer neutrophils and macrophages are recruited towards muscle injury following dexamethasone treatment.

5 dpf *mpx:GFP; fms:mCherry+* larvae were injured and treated with DEX. Larvae were then live imaged on a multi-photon system from 0.5-3.4 hpi with 6.8 minute intervals between frames. Live imaging revealed fewer GFP+ neutrophils and mCherry+ macrophages were recruited towards muscle injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.9. Fewer neutrophils and no macrophages are recruited towards muscle injury following metronidazole treatment.

5 dpf *mpx:GFP; fms:mCherry+* larvae were injured and treated with MTZ. Larvae were then live imaged on a multi-photon system from 0.5-3.4 hpi with 7.3 minute intervals between frames. Live imaging revealed fewer GFP+ neutrophils and no mCherry+ macrophages were recruited towards muscle injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.10. Migratory *Tp1:H2BmCherry+* cells observed following injury in an absence of macrophages.

5 dpf *Tp1:H2BmCherry; fms:mCherry+* larvae were injured and treated with MTZ. Larvae were then live imaged on a multi-photon system from 1-18.3 hpi with 15 minute intervals between frames. Live imaging revealed many migratory mCherry+ cells following injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.11. Recruited neutrophils express *Tp1:H2BmCherry* following injury in an absence of macrophages.

5 dpf *mpx:GFP; Tp1:H2BmCherry; fms:mCherry+* larvae were injured and treated with MTZ. Larvae were then live imaged on a multi-photon system from 1.5-18.3 hpi with 10 minute intervals between frames. Live imaging revealed eGFP+mCherry+ neutrophils were recruited towards muscle injury.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.12. Macrophages are recruited to injured muscle.

5 dpf *fms:mCherry+* larvae were injured and live imaged on a multi-photon system from 1-3.7 hpi with 10 minute intervals between frames. Live imaging demonstrates recruitment of mCherry+ macrophages towards muscle injury. Injury can be visualised in SHG signal.

Scale bar: 50 µm. time stamp in minutes post injury.

Movie 9.13. Fewer macrophages are recruited to muscle injury following DAPT treatment.

5 dpf *fms:mCherry+* larvae were injured and treated with DAPT. Larvae were then live imaged on a multi-photon system from 1-3.5 hpi with 10 minute intervals between frames. Live imaging revealed fewer mCherry+ macrophages were recruited towards muscle injury. Injury can be visualised in SHG signal.

Scale bar: 50 µm. time stamp in minutes post injury.

9.3.2 Excel files

File 9.1. Summary of datasets and significance chapter 3.

Excel workbook containing statistical results and the means and standard deviations from each dataset. Workbook has been separated in function of the figure number. ANOVA results section includes the comparisons made (condition, cell type and anatomic position), the type of ANOVA conducted (Classical or ART) and the p-value. Pair-wise results section includes the comparisons made, the type of pair-wise test (monovariate: Student's t-test, Welch's t-test, Wilcoxon-Mann-Whitney [WMW] test; multivariable: Tukey or Dunn's test with Benjamini and Hochberg (bh) correction) and the p-value. The pair-wise results section also includes results from a normality test (Shapiro–Wilk test) and test for equal variance (Bartlett test, Fligner-Killeen test or F-test). For each worksheet, the mean and standard deviation for each condition has also been listed to understand any significant changes.

File 9.2. Summary of datasets and significance chapter 4.

Excel workbook containing statistical results and the means and standard deviations from each dataset. Workbook has been separated in function of the figure number. ANOVA results section includes the comparisons made (condition, cell type and anatomic position), the type of ANOVA conducted (Classical or ART) and the p-value. Pair-wise results section includes the comparisons made, the type of pair-wise test (monovariate: Student's t-test, Welch's t-test, Wilcoxon-Mann-Whitney [WMW] test; multivariable: Tukey or Dunn's test with Benjamini and Hochberg (bh) correction) and the p-value. The pair-wise results section also includes results from a normality test (Shapiro–Wilk test) and test for equal variance (Bartlett test, Fligner-Killeen test or F-test). For each worksheet, the mean and standard deviation for each condition has also been listed to understand any significant changes.

File 9.3. Summary of datasets and significance chapter 5.

Excel workbook containing statistical results and the means and standard deviations from each dataset. Workbook has been separated in function of the figure number. ANOVA results section includes the comparisons made (condition, cell type and anatomic position), the type of ANOVA conducted (Classical or ART) and the p-value. Pair-wise results section includes the comparisons made, the type of pair-wise test (monovariate: Student's t-test, Welch's t-test, Wilcoxon-Mann-Whitney [WMW] test; multivariable: Tukey or Dunn's test with Benjamini and Hochberg (bh) correction) and the p-value. The pair-wise results section also includes results from a normality test (Shapiro–Wilk test) and test for equal variance (Bartlett test, Fligner-Killeen test or F-test). For each worksheet, the mean and standard deviation for each condition has also been listed to understand any significant changes.