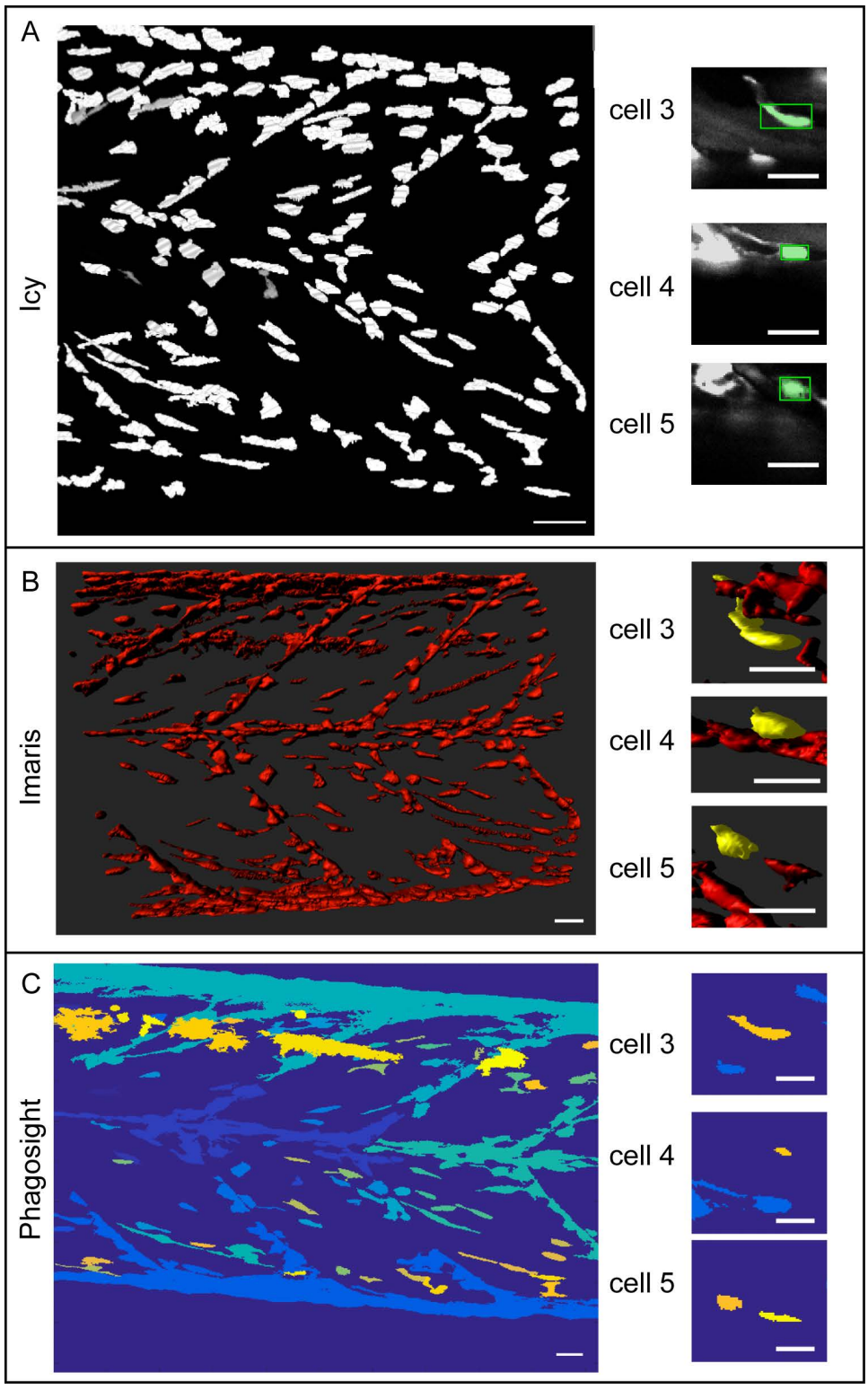

Figure S1

Figure S1: Segmentation of dataset1 by Icy (A), Imaris (B) and Phagosight (C) using optimal parameters as established in Table S1. Cells 3, 4 and 5 are shown for each segmentation method. Scale bar 20µm.

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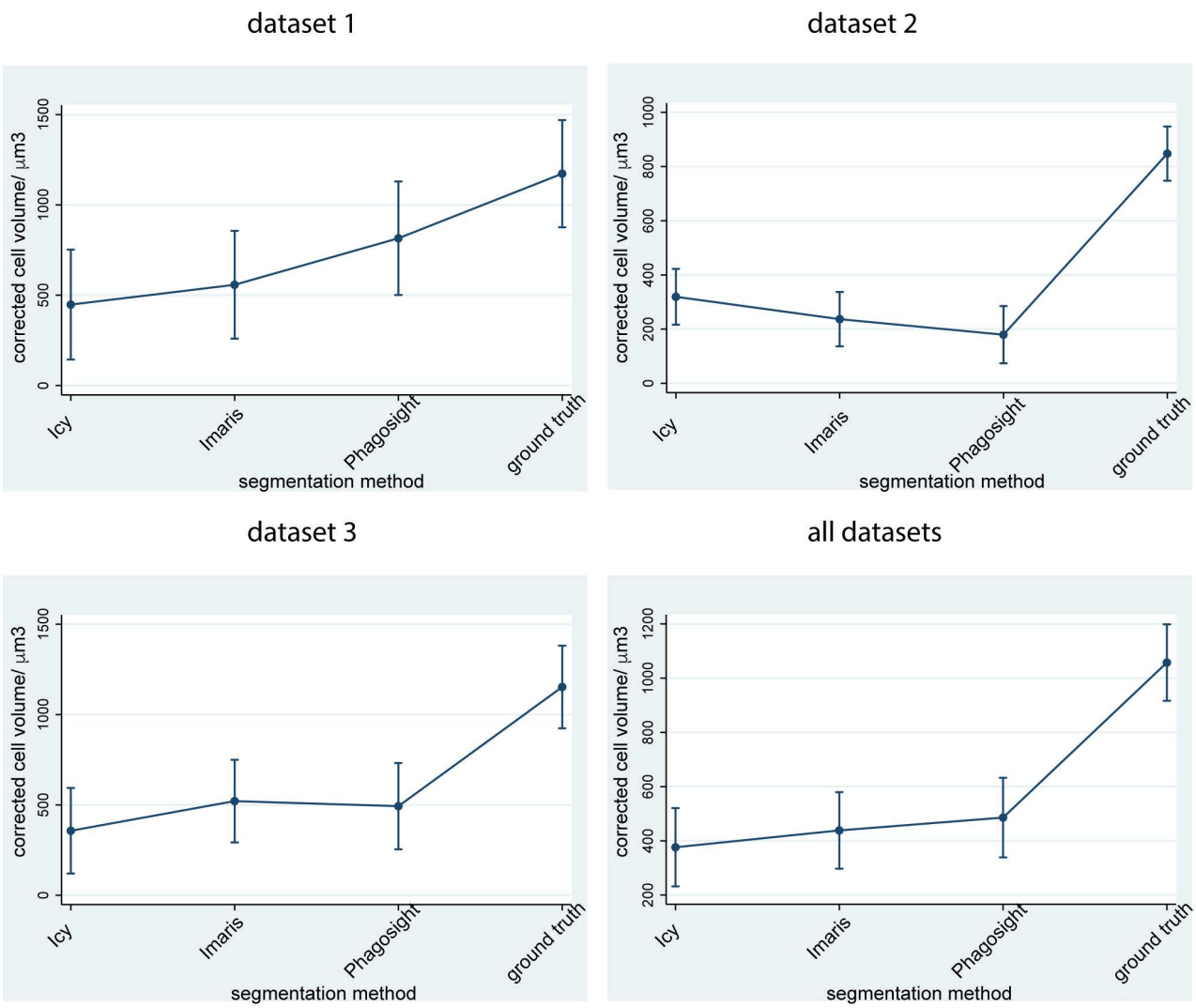

Figure S2

Figure S2: Comparison of the corrected average volumes of the ten selected cells from datasets 1-3 following segmentation by Icy, Imaris and Phagosight relative to manual segmentation. Volumes were calculated using a multiple-level mixed effects linear regression model. 95% confidence intervals (CI) are shown representing the certainty of the predicted volumes. A lack of overlap between CI for two conditions represents a significant difference in their distribution.

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Table S1: Table detailing segmentation parameters applied for optimal identification of cells from datasets 1-3 by Icy, Imaris and Phagosight relative to the ground truth. Parameters for segmentation by Imaris (Threshold, Quality and Number of Voxels, A), Icy (HK Means parameters: Minimum size, Maximum size and class number, B) or Phagosight (Minimum and Maximum size, C) were varied sequentially from default settings and the number of detected cells measured. The difference in the number of objects detected was compared against a manual ground truth of objects counted by eye. Detection of 10 selected cells in the myotome at three selected time-points (equivalent to 30 objects) was used to evaluate the effectiveness of the segmentation. Good detection corresponded to objects observed by manual identification, false detection corresponded to a lack of manual identification and no detection corresponded to a failure for the program to identify an object detected by eye. The optimal segmentation parameters selected are shown in yellow.

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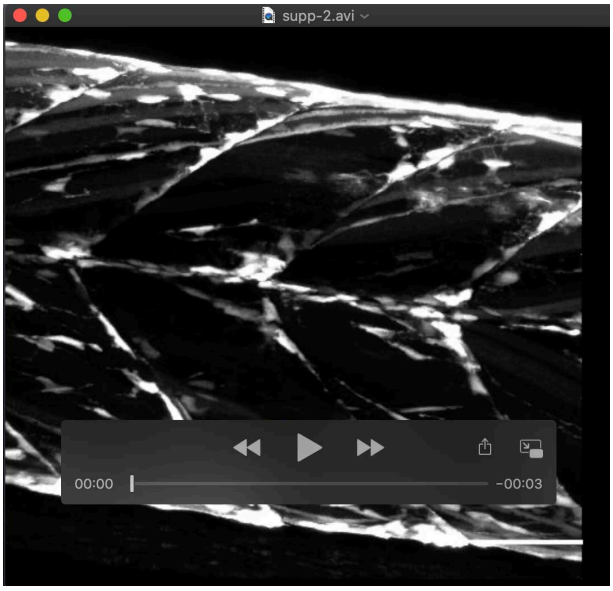
Table S2: Statistical significance of differences in parameters of cell movement and shape between control (DMSO) treated, ROCKout treated and Y-27632 treated larvae is shown by p values. Significance for each parameter between conditions was tested by a Kruskal-Wallis test followed by pairwise comparisons using a Dunn post-hoc test.

	Distance (µm)	Displacement (µm)	Directionality	Directional autocorrelation	Instantaneous speed (µm/hr)	Surface Area (µm^2)	Volume (µm^3)	Sphericity (%)	Convexity (%)	Roundness (%)
Kruskal-Wallis statistic	2.41e-10	5.58e-12	< 2.2e-16	1.52e-8	0.0043	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16
Control-ROCKout	6.6e-10	0.87	3.9e-09	0.00065	0.30	2e-16	1.4e-12	<2e-16	<2e-16	<2e-16
Control-Y27632	7.4e-05	7.2e-11	< 2e-16	0.00079	0.011	2e-16	< 2e-16	<2e-16	<2e-16	0.0034
ROCKout-Y27632	0.0019	3.1e-09	0.00083	1.9e-10	0.0012	1.3e-15	8.4e-08	0.026	0.021	<2e-16

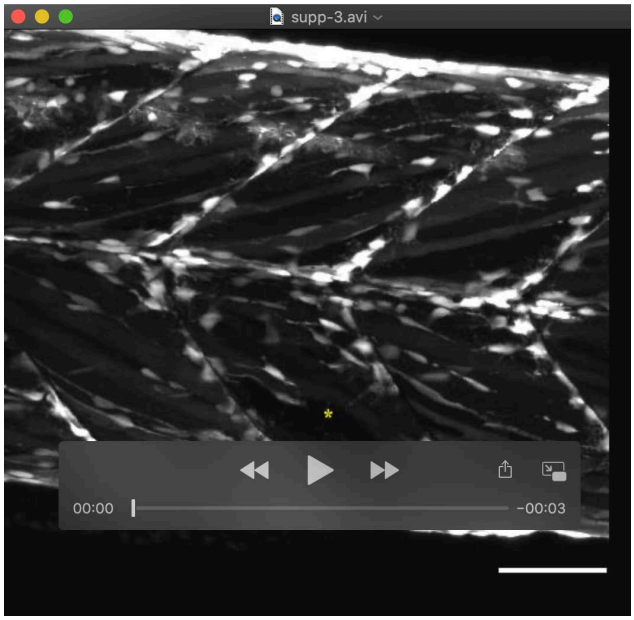
Table S3: Statistical significance of differences in parameters of cell movement and shape between injured and uninjured myotomes of control and blebbistatin treated larvae is shown by p values. Significance for differences in a parameter between conditions was tested by a Kruskal-Wallis (KW) test followed by pairwise comparisons using a Dunn post-hoc test.

	Distance (µm)	Displacement (µm)	Directionality	Directional autocorrelation	Instantaneous speed (µm/hr)	Surface Area (µm^2)	Volume (µm^3)	Sphericity (%)	Convexity (%)	Roundness (%)
KW statistic	1.85e-09	2.42e-10	0.0011	5.65e-05	1.62e-11	0.061	0.20	0.059	0.12	0.32
2-3	1.29e-06	3.18e-05	0.1505	8.4e-05	9.10e-07	0.0098	0.11	0.0038	0.018	0.097
1-3	0.17	0.0264	0.1159	0.0015	0.0531	0.092	0.41	0.023	0.072	0.35
1-2	2.13e-10	2.57e-11	0.0068	0.6305	7.97e-13	0.13	0.13	0.23	0.24	0.16
0-3	0.11	0.0171	0.0120	1.1e-05	0.16	0.0078	0.038	0.024	0.014	0.39
0-2	3.31e-05	0.0131	0.0913	0.5269	5.94e-06	0.50	0.29	0.20	0.48	0.036
0-1	0.0065	2.38e-06	0.0001	0.2026	0.0016	0.12	0.044	0.47	0.22	0.22

Groups
0: -blebbistatin, -injury
1: -blebbistatin, +injury
2: +blebbistatin, -injury
3: +blebbistatin, +injury



Movie 1: maximum intensity projection of a confocal imaging time-lapse sequence of a 7 dpf pax7a:egfp larvae that has been injured (arrowhead) in the ventral myotome (dataset 1). Z-stacks were acquired every 20 minutes from 8 hours after injury (asterisk). Scale bar 50µm.



Movie 2: maximum intensity projection of a confocal imaging time-lapse sequence of a 7 dpf pax7a:egfp larvae that has been injured (arrowhead) in the ventral myotome (dataset 3). Z-stacks were acquired every 20 minutes from 8 hours after injury (asterisk). Scale bar 50µm.

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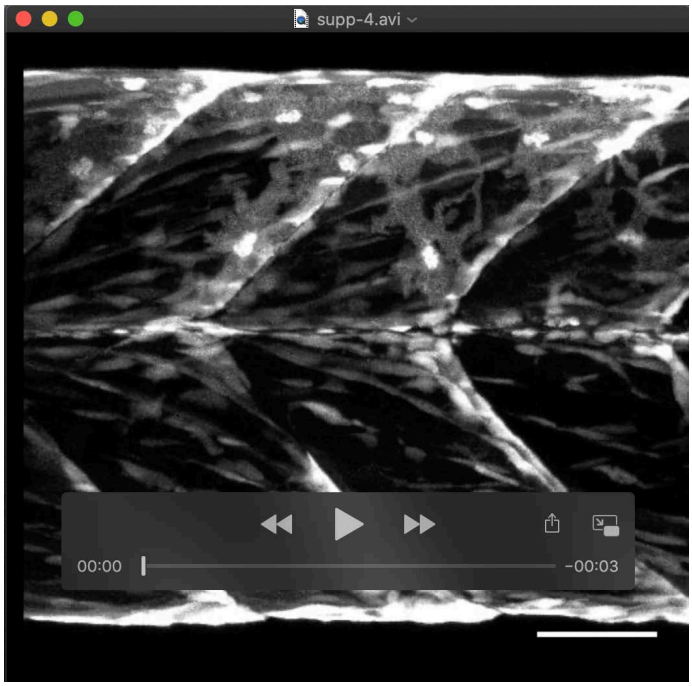
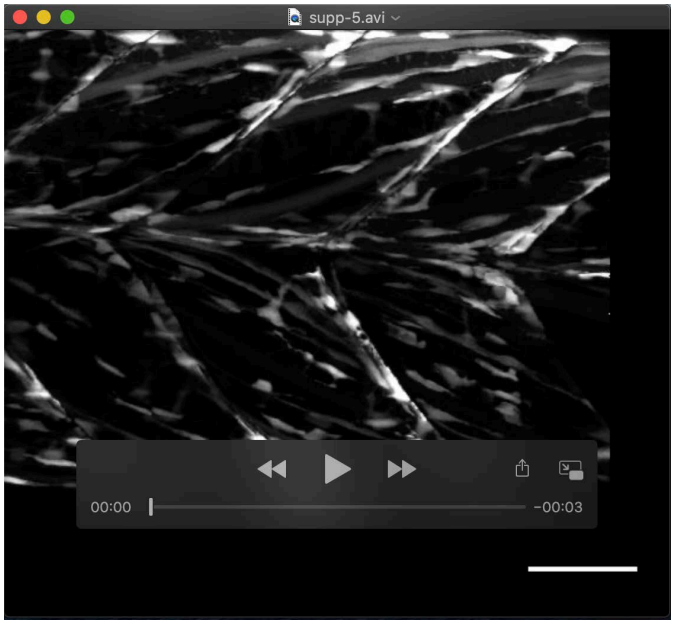


Movie 3: maximum intensity projection of a confocal imaging time-lapse sequence of an uninjured 3 dpf pax7a:egfp larvae treated with 1% DMSO (dataset 4). Z-stacks were acquired every 20 minutes. Scale bar 50µm.



Movie 4: maximum intensity projection of a confocal imaging time-lapse sequence of an uninjured 3 dpf pax7a:egfp larvae treated with 15µM Y-27632 (dataset 5). Z-stacks were acquired every 20 minutes. Scale bar 50µm.

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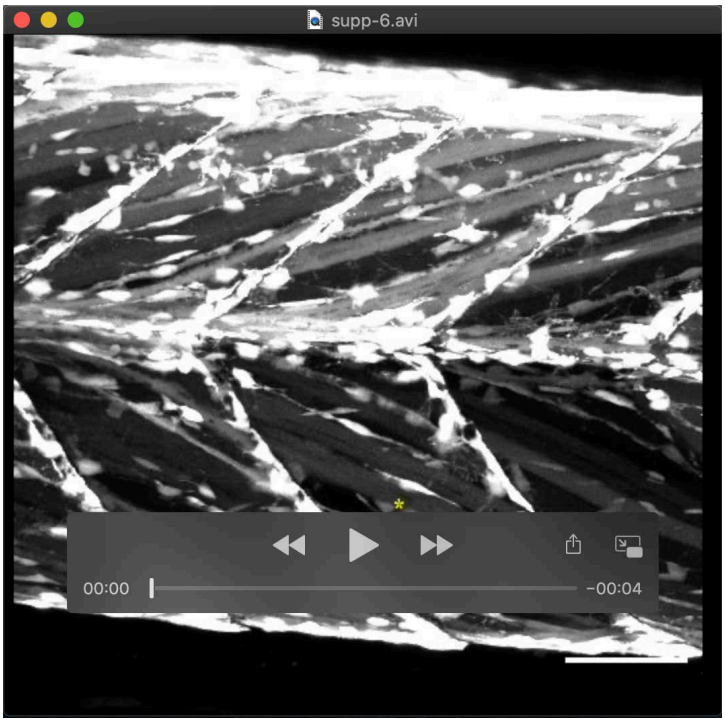

Movie 5: maximum intensity projection of a confocal imaging time-lapse sequence of a pax7a:egfp larvae that has been injured (asterisk) and treated with 0.8µM blebbistatin (dataset 10). Scale bar 50µm.

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