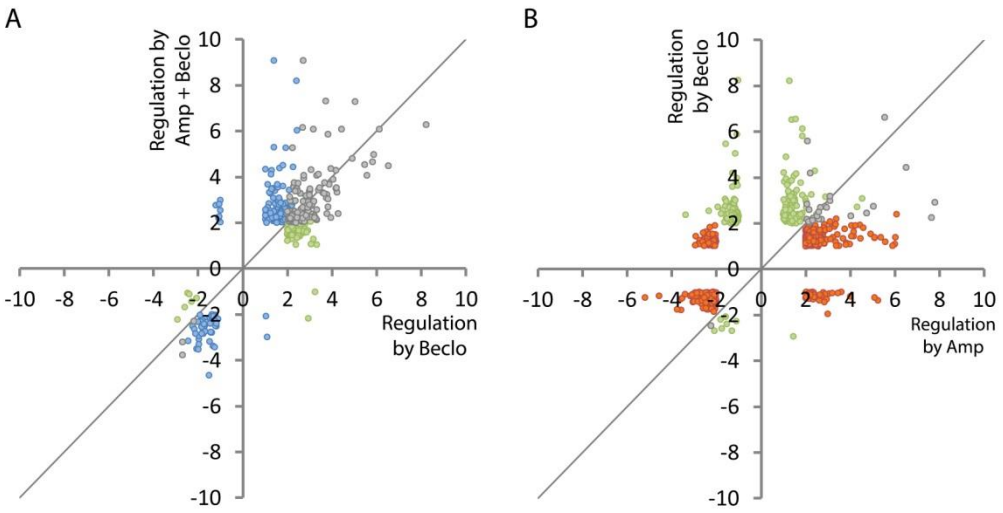


Figure S1. Scatter plot showing the difference and overlaps in gene regulation between different treatments. A. Comparison between beclomethasone and combined beclomethasone and amputation treatment. For all genes showing significant regulation upon beclomethasone (green and grey dots) or the combined beclomethasone and amputation treatment (blue and grey dots), the fold change due to beclomethasone and amputation treatment was plotted as a function of the fold change due to beclomethasone. The plot shows a large group of genes (grey dots) regulated by both treatments. B. Comparison between amputation and beclomethasone treatment. For all genes showing significant regulation upon amputation (red and grey dots) or beclomethasone treatment (green and grey dots), the fold change due to beclomethasone treatment was plotted as a function of the fold change due to amputation. The plot shows a very small group of genes (grey dots) regulated by both treatments. The grey line indicates the point at which the two different treatment have the same effect. Significantly regulated genes were selected by using a p.adj<0.05 and |FoldChange|>2 cutoff.

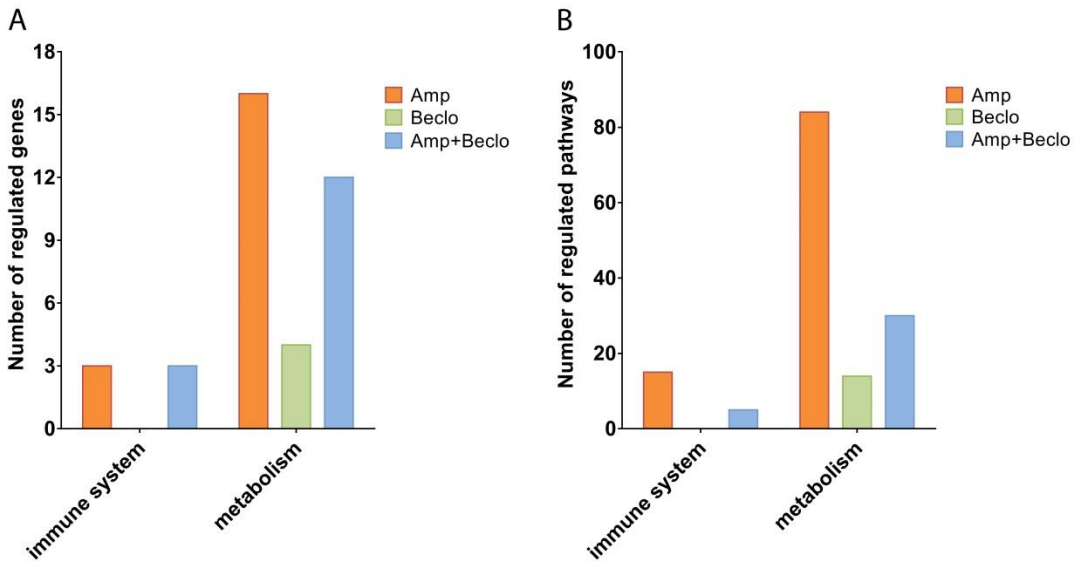
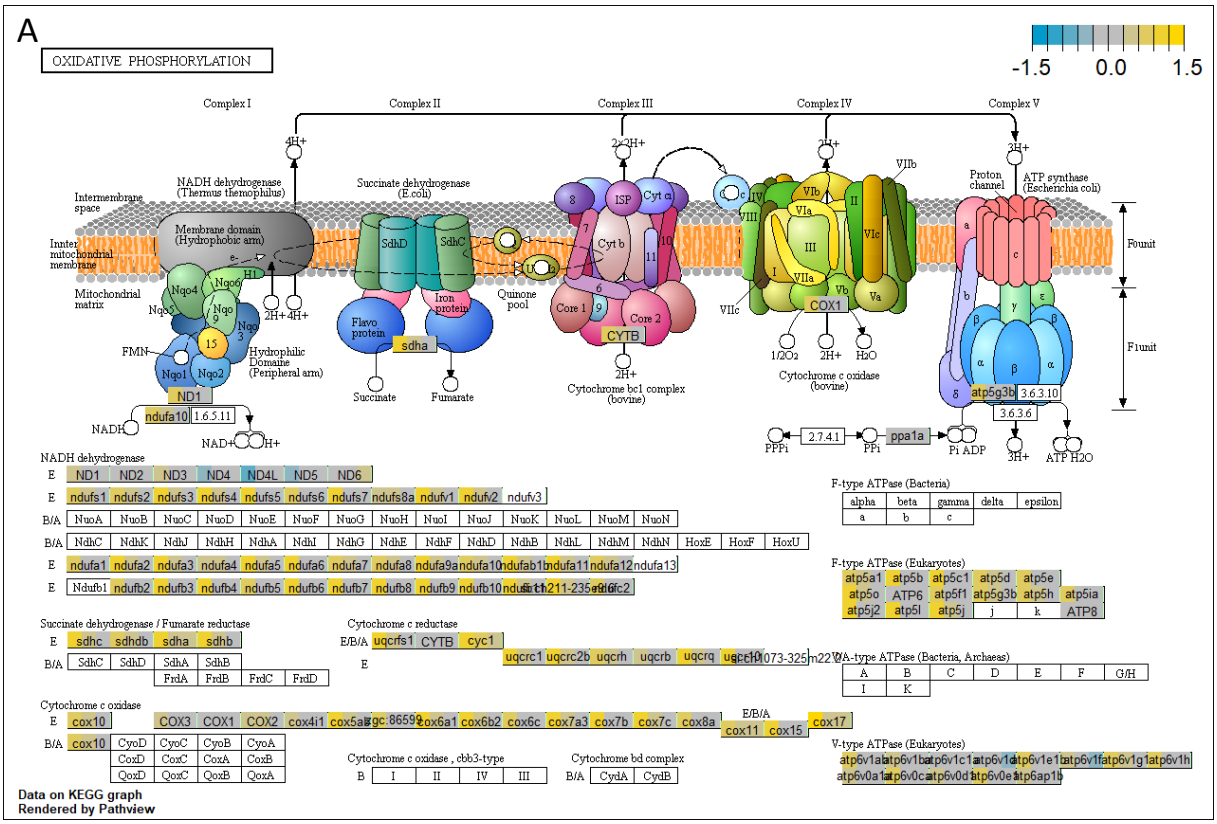
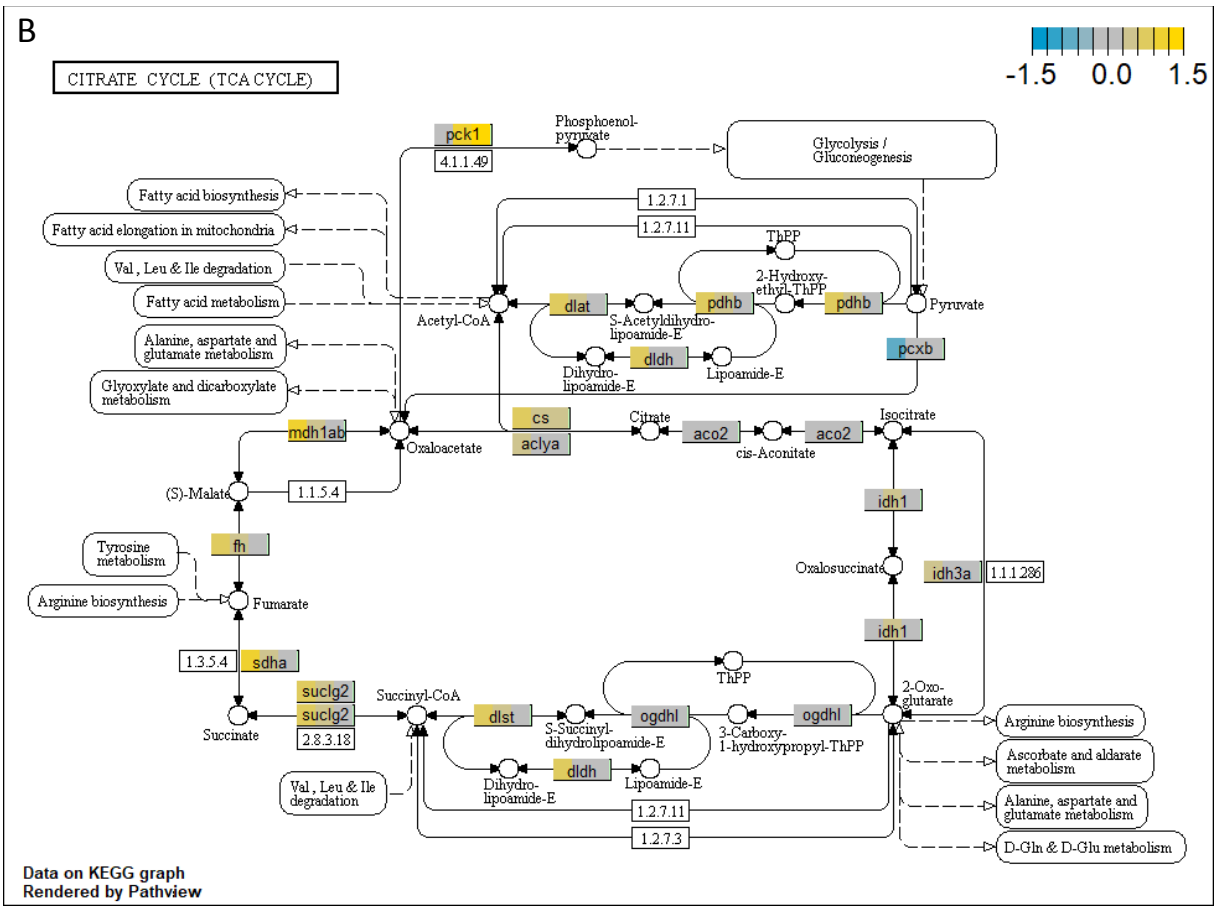


Figure S2. Gene ontology analysis of RNA sequencing experiment. A. The number of KEGG pathways overrepresented in clusters of genes significantly regulated by amputation, beclomethasone, and amputation+beclomethasone. Amputation regulated pathways involved in the immune system and in metabolism. Amputation+beclomethasone treatment regulated a lower number of regulated pathways involved in both the immune system and metabolism. B. The number of regulated genes involved in the overrepresented pathways. Amputation regulated genes involved in immune- and metabolism-related pathways. Amputation+beclomethasone regulated a lower number of genes involved in these pathways.


A
OXIDATIVE PHOSPHORYLATION
-1.5 0.0 1.5
Complex I
Complex II
Complex III
Complex IV
Complex V
NADH dehydrogenase (Thermus thermophilus)
Succinate dehydrogenase (E.coli)
Cytochrome bc1 complex (bovine)
Cytochrome c oxidase (bovine)
Proton channel
ATP synthase (Escherichia coli)
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Membrane domain (Hydrophobic arm)
Hydrophilic Domain (Peripheral arm)
Quinone pool
Flavo protein
Iron protein
Succinate
Fumarate
NADH
NAD+
FMN
Fo unit
F1 unit
NADH dehydrogenase
Succinate dehydrogenase / Fumarate reductase
Cytochrome c reductase
Cytochrome c oxidase
Cytochrome c oxidase , cbb3-type
Cytochrome bd complex
F-type ATPase (Bacteria)
F-type ATPase (Eukaryotes)
V/A-type ATPase (Bacteria, Archaeas)
V-type ATPase (Eukaryotes)
Data on KEGG graph
Rendered by Pathview


B
CITRATE CYCLE (TCA CYCLE)
-1.5 0.0 1.5
pck1
4.1.1.49
Phosphoenol-pyruvate
Glycolysis / Gluconeogenesis
Fatty acid biosynthesis
Fatty acid elongation in mitochondria
Val , Leu & Ile degradation
Fatty acid metabolism
Alanine, aspartate and glutamate metabolism
Glyoxylate and dicarboxylate metabolism
1.2.7.1
1.2.7.11
ThPP
2-Hydroxy-ethyl-ThPP
dlat
pdhb
S-Acetyldihydro-lipoamide-E
Dihydro-lipoamide-E
dldh
Lipoamide-E
Acetyl-CoA
Pyruvate
pcxb
mdh1ab
Oxaloacetate
cs
aclya
Citrate
aco2
cis-Aconitate
Isocitrate
idh1
(S)-Malate
1.1.5.4
Tyrosine metabolism
fh
Arginine biosynthesis
Fumarate
Oxalosuccinate
idh3a
1.1.1.286
1.3.5.4
sdha
suclg2
suclg2
2.8.3.18
Succinate
Succinyl-CoA
dlst
S-Succinyl-dihydrolipoamide-E
ogdhl
3-Carboxy-1-hydroxypropyl-ThPP
2-Oxo-glutarate
Arginine biosynthesis
Ascorbate and aldarate metabolism
Alanine, aspartate and glutamate metabolism
D-Gln & D-Glu metabolism
Val , Leu & Ile degradation
1.2.7.11
1.2.7.3
Data on KEGG graph
Rendered by Pathview

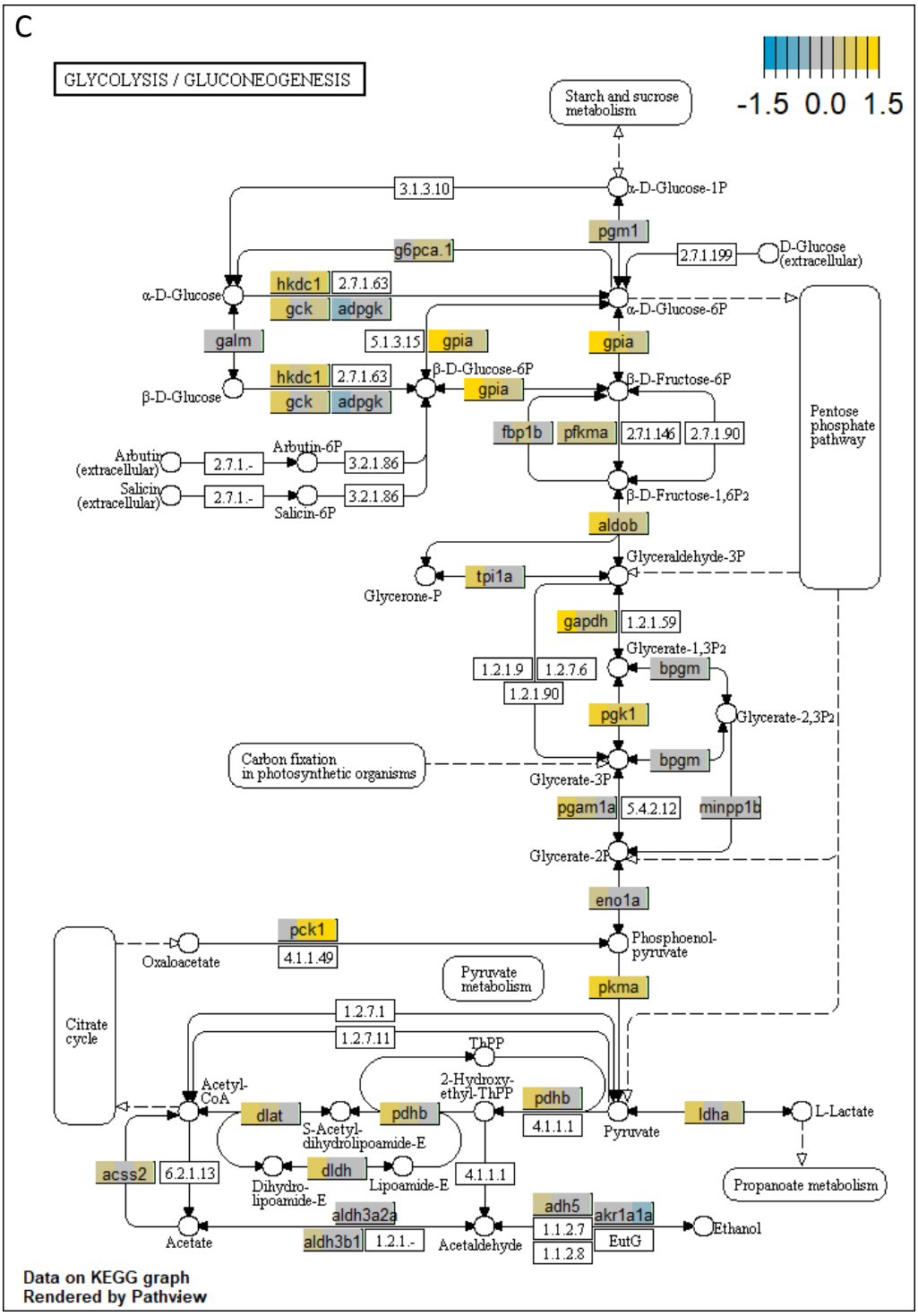


Figure S3. Significantly enriched KEGG pathways mapped with gene expression level. In the oxidative phosphorylation (A), Citrate cycle (TCA cycle) (B) and Glycolysis/ Gluconeogenesis (C) pathways, the fold change of the genes is indicated by different colour and the intensity of the colour as shown in the scale. The three colour shown in one gene indicated the logarithm fold change of amputation, beclomethasone and amputation+beclomethasone treatment respectively. The maps show that the vast majority of the amputation-induced changes in gene expression are inhibited by beclomethasone treatment in these pathways.

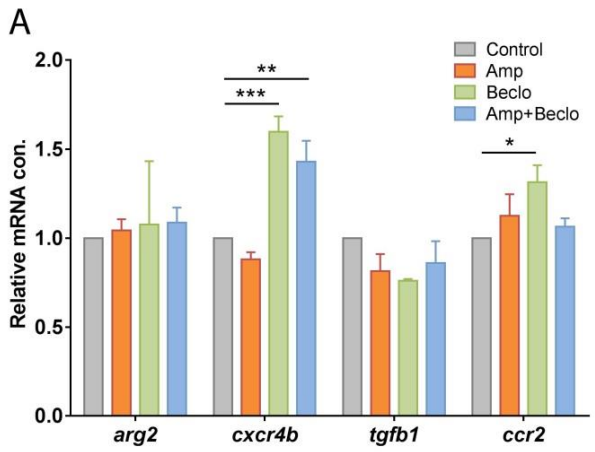


Figure S4. Expression levels of immune-related genes in FACS-sorted macrophages, determined by qPCR for *arg2, cxcr4b, tgfb1, ccr2* (A) at 24hpa in 3 dpf larvae. The expression level of *cxcr4b* was increased by beclomethasone treatment. Statistical analysis were performed by ANOVA with a Fisher's LSD post hoc. Values shown are the means ± s.e.m. of three independent experiments. Statistical significance is indicated by: * P<0.05; ** P<0.01; *** P<0.001.

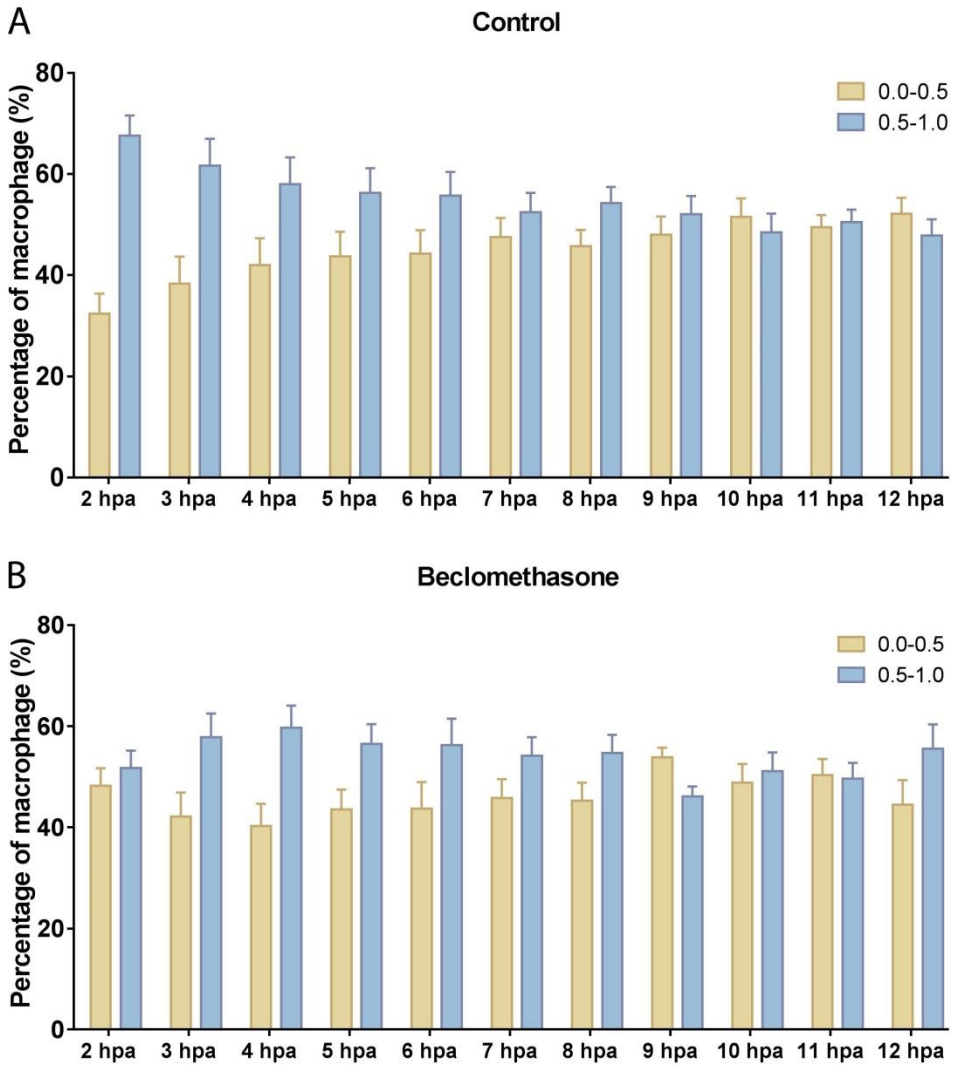


Figure S5. Effect of beclomethasone on the morphology of macrophages. The percentage of macrophages with different levels of circularity (0.0-0.5 and 0.5-1.0) were determined over time for the control group (A) and beclomethasone-treated group (B). In the control group, an increase in the percentage of macrophages with a circularity level between 0.0 and 0.5 and a decrease in the percentage of macrophages with a level between 0.5 and 1.0 was observed. In the beclomethasone-treated group, these changes were not observed. Values shown are means ± s.e.m..

Table S1. KEGG-pathways, based on RNA sequencing analysis.

Amputation

Term	Count	%	P-value	Fold Enrichment
Immune system				
NOD-like receptor signaling pathway	6	1.03	2.89E-02	3.43
Toll-like receptor signaling pathway	8	1.37	6.13E-02	2.26
Cytokine-cytokine receptor interaction	11	1.89	6.58E-02	1.88
Jak-STAT signaling pathway	8	1.37	9.53E-02	2.04
Metabolism				
Oxidative phosphorylation	28	4.81	2.08E-13	5.60
Carbon metabolism	23	3.95	2.72E-10	5.15
Biosynthesis of antibiotics	31	5.33	4.11E-10	3.73
Metabolic pathways	83	14.26	1.20E-09	1.84
Glycolysis / Gluconeogenesis	17	2.92	3.07E-09	6.52
Biosynthesis of amino acids	13	2.23	3.87E-05	4.28
Arginine and proline metabolism	10	1.72	1.33E-04	5.00
Pentose phosphate pathway	7	1.20	6.51E-04	6.32
Citrate cycle (TCA cycle)	6	1.03	5.67E-03	5.09
Fructose and mannose metabolism	6	1.03	1.42E-02	4.10
Pyruvate metabolism	6	1.03	1.73E-02	3.91
Galactose metabolism	5	0.86	2.82E-02	4.24
Insulin resistance	10	1.72	4.62E-02	2.11
Glycine, serine and threonine metabolism	5	0.86	7.43E-02	3.11
Propanoate metabolism	4	0.69	7.54E-02	4.00
RNA degradation	7	1.20	8.06E-02	2.31
Others				
Cardiac muscle contraction	13	2.23	1.30E-04	3.79

Amputation & Beclomethasone

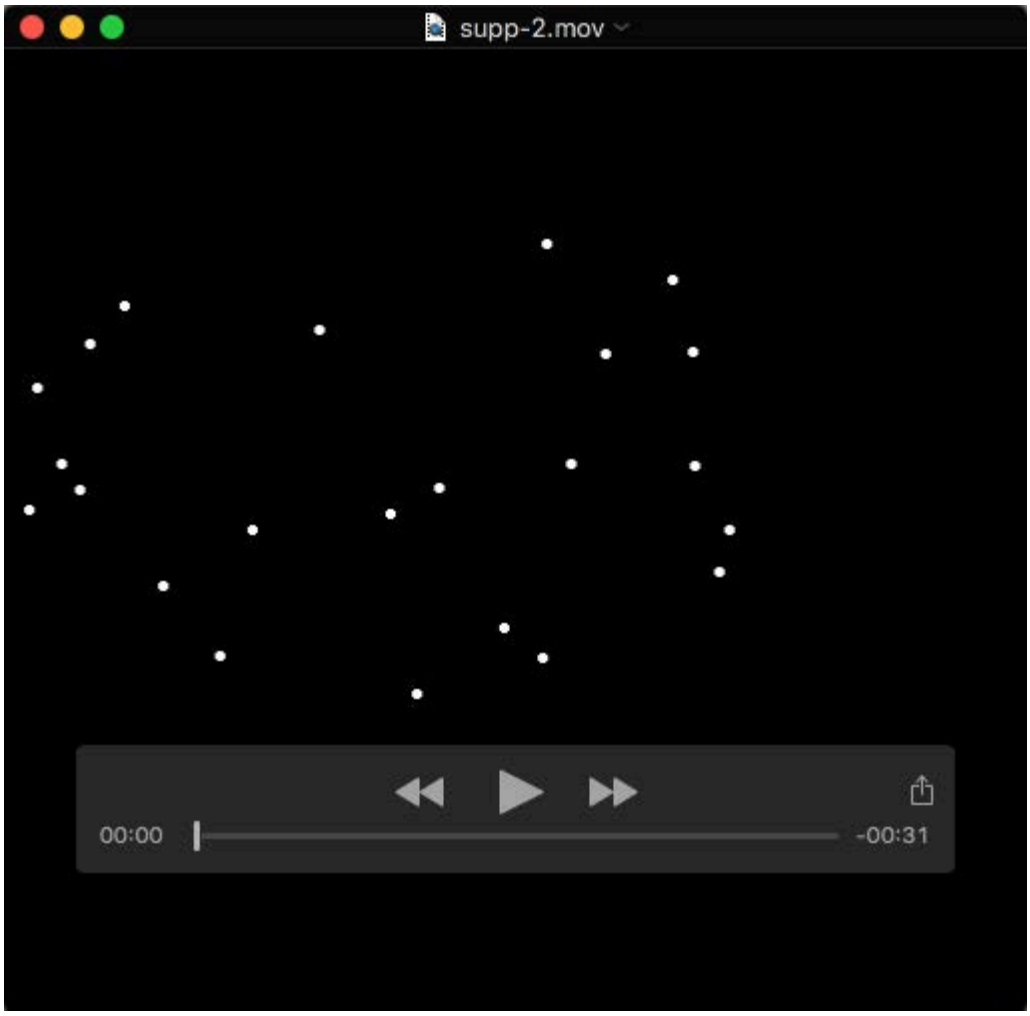
Term	Count	%	P-value	Fold Enrichment
Immune system				
Toll-like receptor signaling pathway	6	1.97	8.62E-03	4.64
Metabolism				
Insulin signaling pathway	10	3.28	3.19E-04	4.45
Starch and sucrose metabolism	5	1.64	6.92E-04	11.96
Insulin resistance	8	2.62	1.49E-03	4.60
Steroid hormone biosynthesis	4	1.31	1.16E-02	8.27
FoxO signaling pathway	7	2.30	2.16E-02	3.15
Biosynthesis of antibiotics	8	2.62	2.94E-02	2.63
Arginine biosynthesis	3	0.98	3.76E-02	9.57
Glyoxylate and dicarboxylate metabolism	3	0.98	6.69E-02	6.96
Galactose metabolism	3	0.98	6.69E-02	6.96
Glycolysis / Gluconeogenesis	4	1.31	6.72E-02	4.19
Retinol metabolism	3	0.98	8.94E-02	5.89
Alanine, aspartate and glutamate metabolism	3	0.98	9.33E-02	5.74
Others				
Adipocytokine signaling pathway	5	1.64	2.43E-02	4.45

Beclomethasone

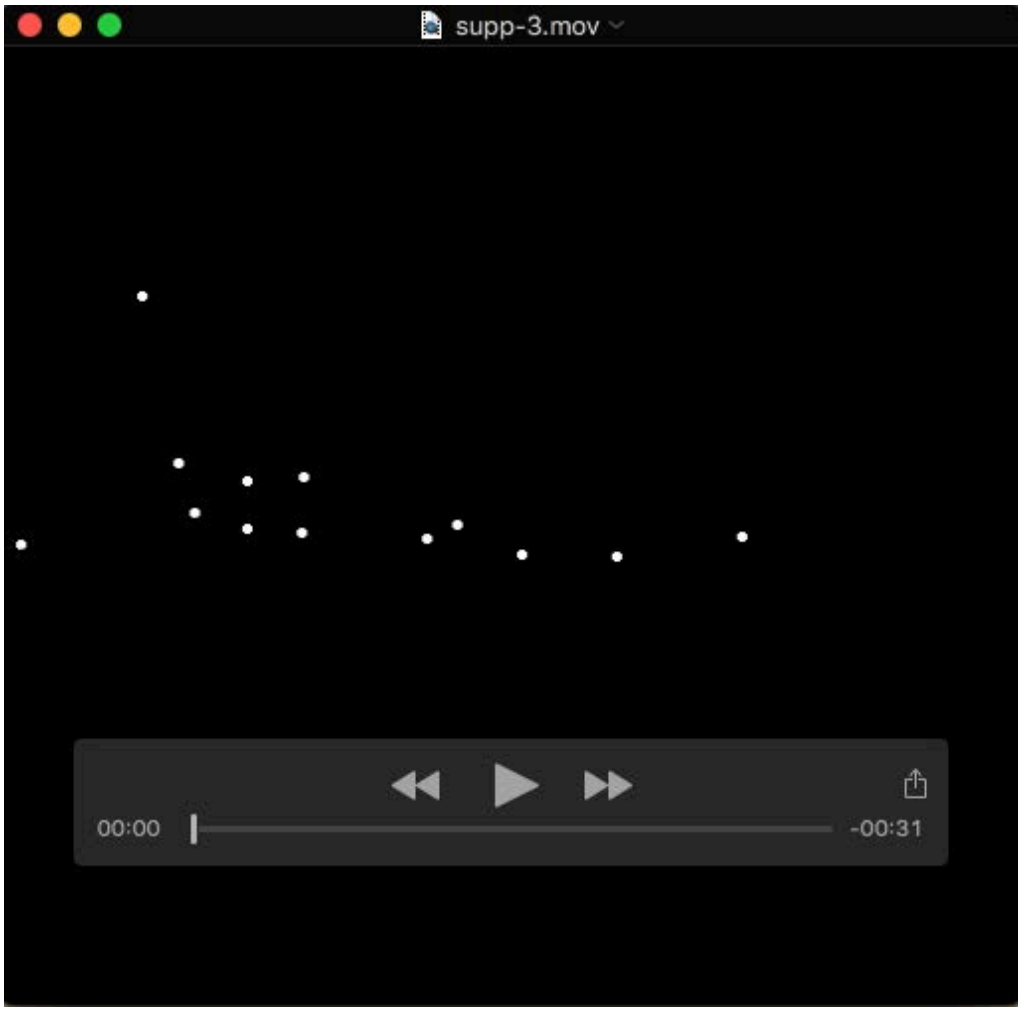
Term	Count	%	P-value	Fold Enrichment
Metabolism				
FoxO signaling pathway	8	4.08	1.48E-03	4.57
Insulin signaling pathway	8	4.08	1.59E-03	4.51
Insulin resistance	6	3.06	1.06E-02	4.38
Glyoxylate and dicarboxylate metabolism	3	1.53	4.35E-02	8.82
Steroid hormone biosynthesis	3	1.53	5.35E-02	7.87
Others				
Focal adhesion	7	3.57	3.80E-02	2.74
Adipocytokine signaling pathway	4	2.04	5.57E-02	4.51

Table S2. Sequences of Primers used in qPCR reactions.

Gene name	Gene accession	Sequence (5'-3')
ppail	ENSDARG00000103994	**Fw:** CATCCACAACCTTCCCGAACAC
		Rv: ACACTGAAACACGGAGGCAAAG
ccl2	ENSDARG00000041835	**Fw:** GTCTGGTGCTCTTCGCTTTC
		Rv: TGCAGAGAAGATGCGTCGTA
cxcl11aa	ENSDARG00000100662	**Fw:** ACTCAACATGGTGAAGCCAGTGCT
		Rv: CTTCAGCGTGGCTATGACTTCCAT
il8	ENSDARG00000104795	**Fw:** TGTGTTATTGTTTTCCTGGCATTTC
		Rv: GCGACAGCGTGGATCTACAG
cxcl18b	ENSDARG00000075045	**Fw:** TCTTCTGCTGCTGCTTGCGGT
		Rv: GGTGTCCCTGCGAGCACGAT
il6	ENSDARG00000102318	**Fw:** CGCTAAGGCAACTGGAAGAC
		Rv: CCAGACCACTGGGAAACACT
il1b	ENSDARG00000098700	**Fw:** TGTGTGTTTGGGAATCTCCA
		Rv: CTGATAAACCAACCGGGACA
tnfa	ENSDARG00000009511	**Fw:** ACCAGGCCTTTTCTTCAGGT
		Rv: TTTGCCTCCGTAGGATTCAG
mmp9	ENSDARG00000042816	**Fw:** CATTAAAGATGCCCTGATGTATCCC
		Rv: AGTGGTGGTCCGTGGTTGAG
arg2	ENSDARG00000039269	**Fw:** AAGGCCATTCTCAGCAGTGT
		Rv: AGGTTTCCCGAAGGTGAAGT
cxcr4b	ENSDART00000061499	**Fw:** GCGACCTCTCAGTCAGCAAT
		Rv: TCACAAGCACCACAAGTCCA
tgfb1	ENSDARG00000041502	**Fw:** CAACCGCTGGCTCTCATTTGA
		Rv: ACAGTCGCAGTATAACCTCAGCT
ccr2	ENSDARG00000079829	**Fw:** TGGCAACGCAAAGGCTTTCAGTGA
		Rv: AGGTTTCCCGAAGGTGAAGT



Movie 1. Macrophage migration upon tail amputation. Tracks of macrophages migrating between 1.5 and 5.5 hpa in 3 dpf larvae in the vehicle-treated group. Confocal microscopy images were analyzed using ImageJ with custom-made plugins, developed by Dr. Joost Willemse (Leiden University), for localization and tracking of cells.



Movie 2. Neutrophil migration upon tail amputation. Tracks of neutrophils migratimg between 1.5 and 5.5 hpa in 3 dpf larvae in the vehicle-treated group. Confocal microscopy images were analyzed using ImageJ with custom-made plugins, developed by Dr. Joost Willemse (Leiden University), for localization and tracking of cells.