Supplemental Information:

An anti-inflammatory NOD-like receptor is required for microglia development

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This file contains:

Supplemental Figures and Table

Figure S1:

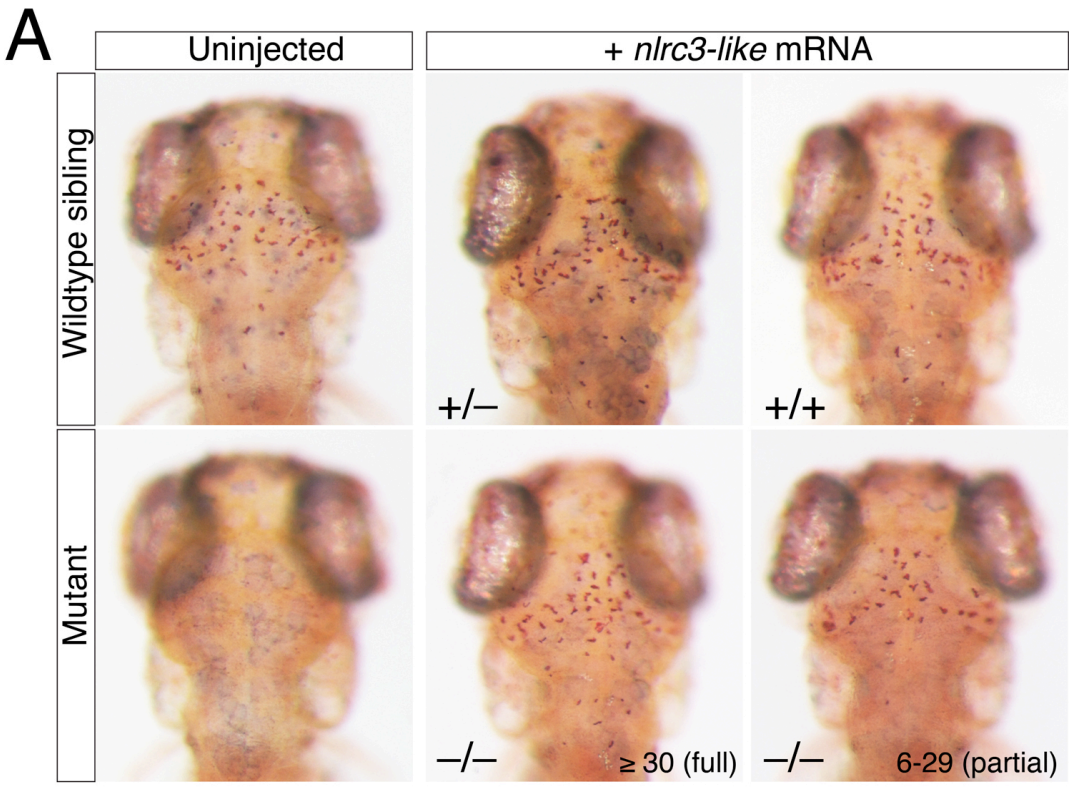

Figure S1. Injection of full-length wildtype *nlrc3-like* mRNA can fully rescue microglia in *nlrc3-like*$^{-/-}$ mutants, Related to Figure 1.

(**A**) Images of embryos injected with synthetic *nlrc3-like* mRNA at the 1-4 cell stage and uninjected controls. At 4–5 dpf, neutral red staining showed that introduction of synthetic *nlrc3-like* mRNA can fully (≥ 30 microglia) or partially (6–29 microglia) rescue the *nlrc3-like* mutants; there was no change in microglia formation in injected heterozygous or wildtype siblings. Dorsal views; anterior to the top. (**B**) Graph showing frequency of the different classes of microglia number (0–5, 6–29, or ≥ 30 microglia) in the indicated genotypes in control (uninjected or water injected) and *nlrc3-like* mRNA injected groups. Number below bar graph represents n, number of embryos analyzed.

Figure S2:

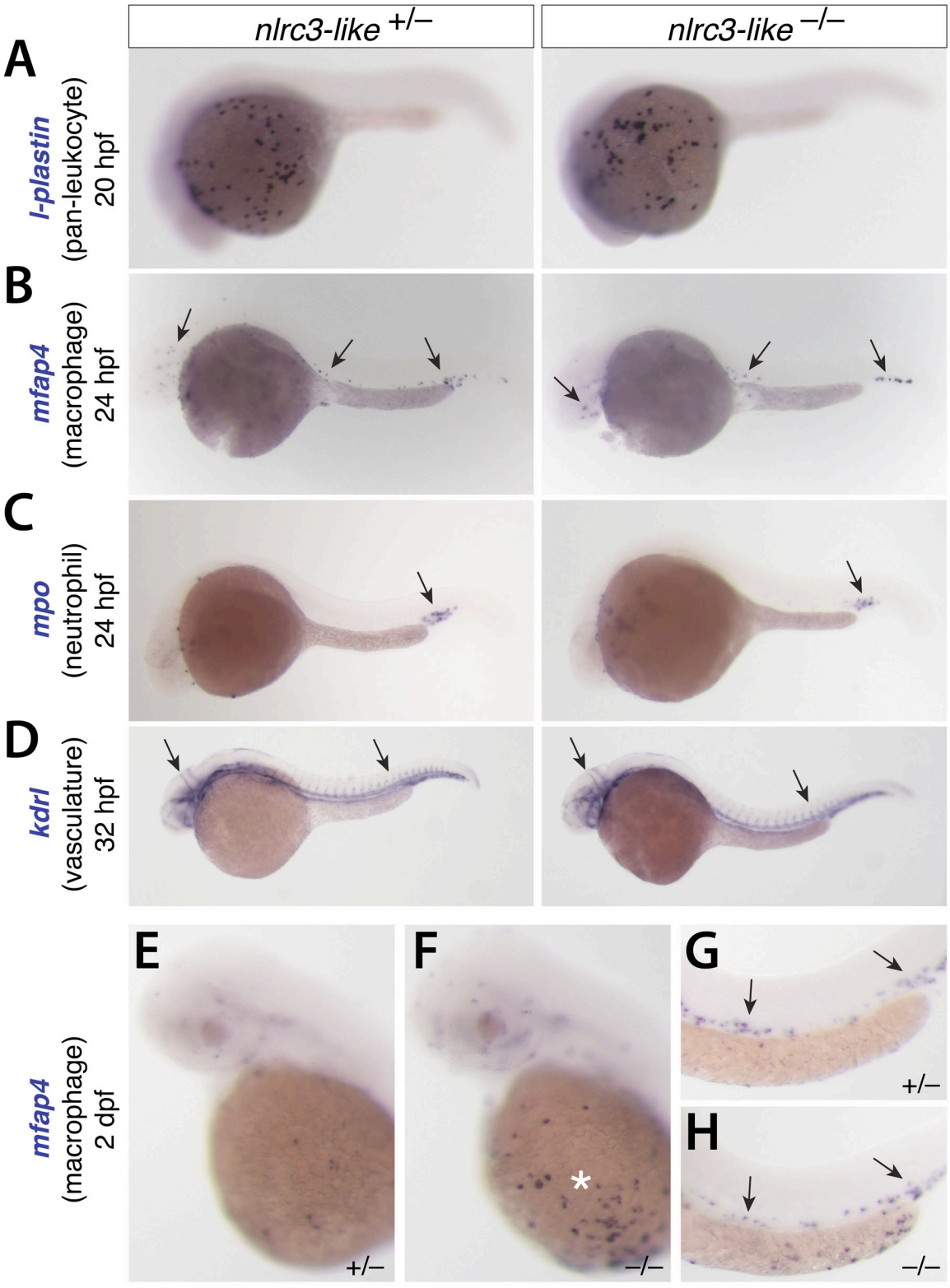
nlrc3-like +/–
nlrc3-like –/–
A
l-plastin
(pan-leukocyte)
20 hpf
B
mfap4
(macrophage)
24 hpf
C
mpo
(neutrophil)
24 hpf
D
kdrl
(vasculature)
32 hpf
mfap4
(macrophage)
2 dpf
E
F
G
H
+/–
–/–
+/–
–/–

Figure S2. Early formation of primitive macrophages, neutrophils, and the vasculature appear normal in *nlrc3-like*$^{-/-}$ mutants, Related to Figure 2.

The images show embryos analyzed by whole mount in situ hybridization. (**A**) At 20 hpf there was no apparent difference in expression in *l-plastin*, a marker of all leukocytes. (**B**) At 24 hpf, *mfap4* expression indicated normal formation and migration of primitive macrophages into the embryo proper in mutants and siblings (arrows). (**C**) At 24 hpf, *mpo* expression showed no difference (arrow) between mutants and siblings. (**D**) Vasculature formed normally in the mutants, based on the expression pattern of the endothelial marker *kdrl* at 32 hpf (arrows). (**E–H**) *mfap4* expression at 2 dpf shows the abundance of macrophages in the periphery of *nlrc3-like* mutants. Compared with heterozygous siblings (**E**), mutants (**F**) have abnormal aggregations of macrophages in the yolk sac (asterisk), but similar pattern of macrophages in the caudal vein (**G–H**, arrows). All panels show lateral views, with anterior to the left and dorsal to the top.

Figure S3:

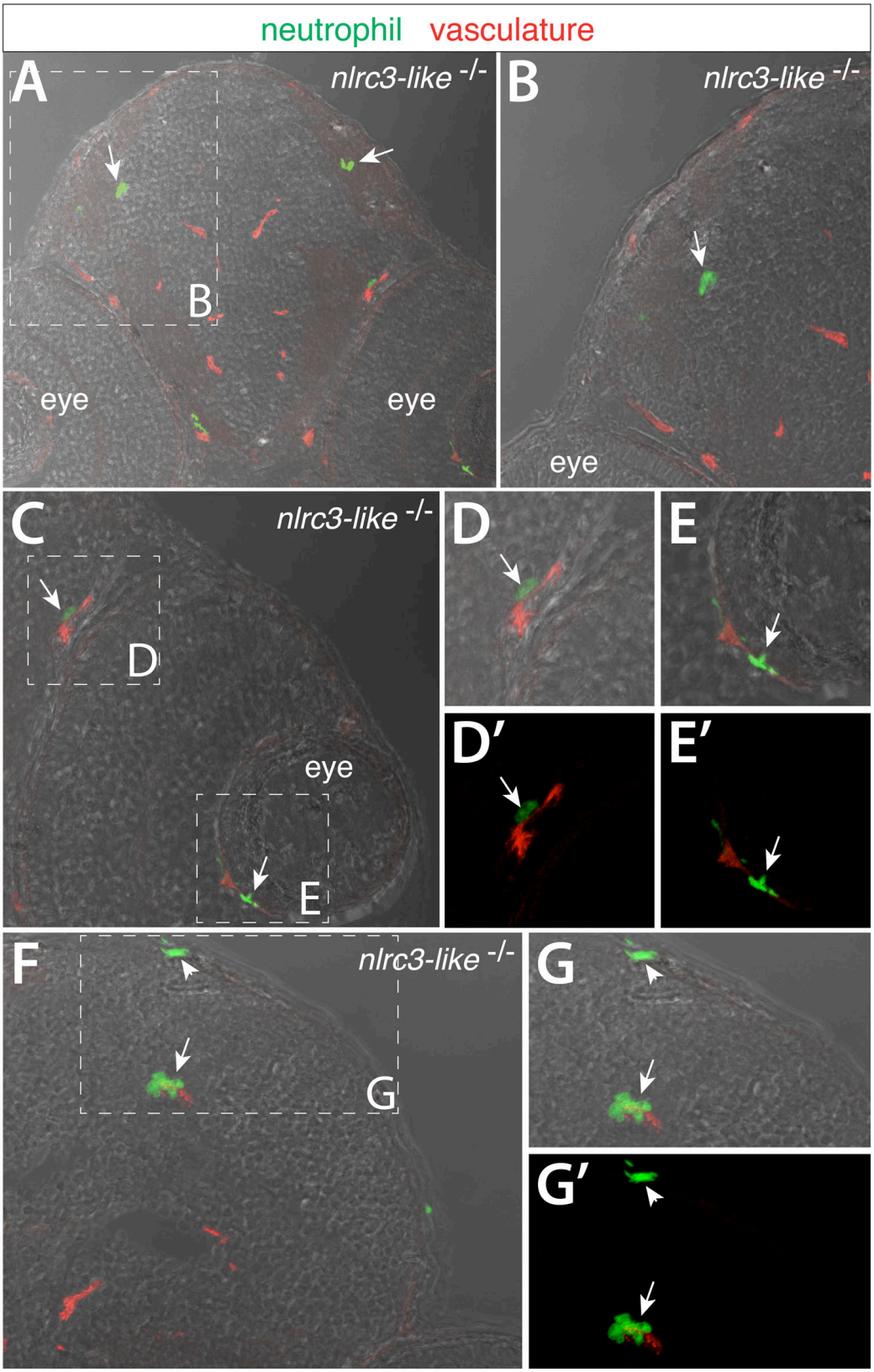
neutrophil vasculature
A
nlrc3-like -/-
B
eye
eye
B
nlrc3-like -/-
eye
C
nlrc3-like -/-
D
eye
E
D
E
D′
E′
F
nlrc3-like -/-
G
G
G′

Figure S3. Transverse sections through the head of *nlrc3-like*$^{-/-}$ embryos at 2.5 dpf show neutrophils that have infiltrated the brain parenchyma and other locations, Related to Figure 4.

Cryosections through the head of double transgenic *nlrc3-like* mutant embryos labeled by neutrophil reporter *lyz:EGFP* and vasculature marker *kdrl:mCherry-CAAX*. Images show overlay of GFP and mCherry expression on the bright field image. (**A**) In *nlrc3-like* mutants, *lyz:EGFP* + neutrophils are found intermixed with cells in the brain parenchyma independent of the vasculature in red (arrows). (**B**) Higher magnification of **A** as demarcated by the white dotted box. Neutrophils in the mutant are also often found within or surrounding eye vasculature (**C–E**). (**D–E**) Higher magnification of the boxed regions in **C**. (**D'–E'**) Red and green fluorescent overlay of the same image as **D–E** respectively. (**F–G**) Neutrophils are also associated with brain vessels (arrow) and in or near the dorsal head epithelium (arrowhead).

Figure S4:

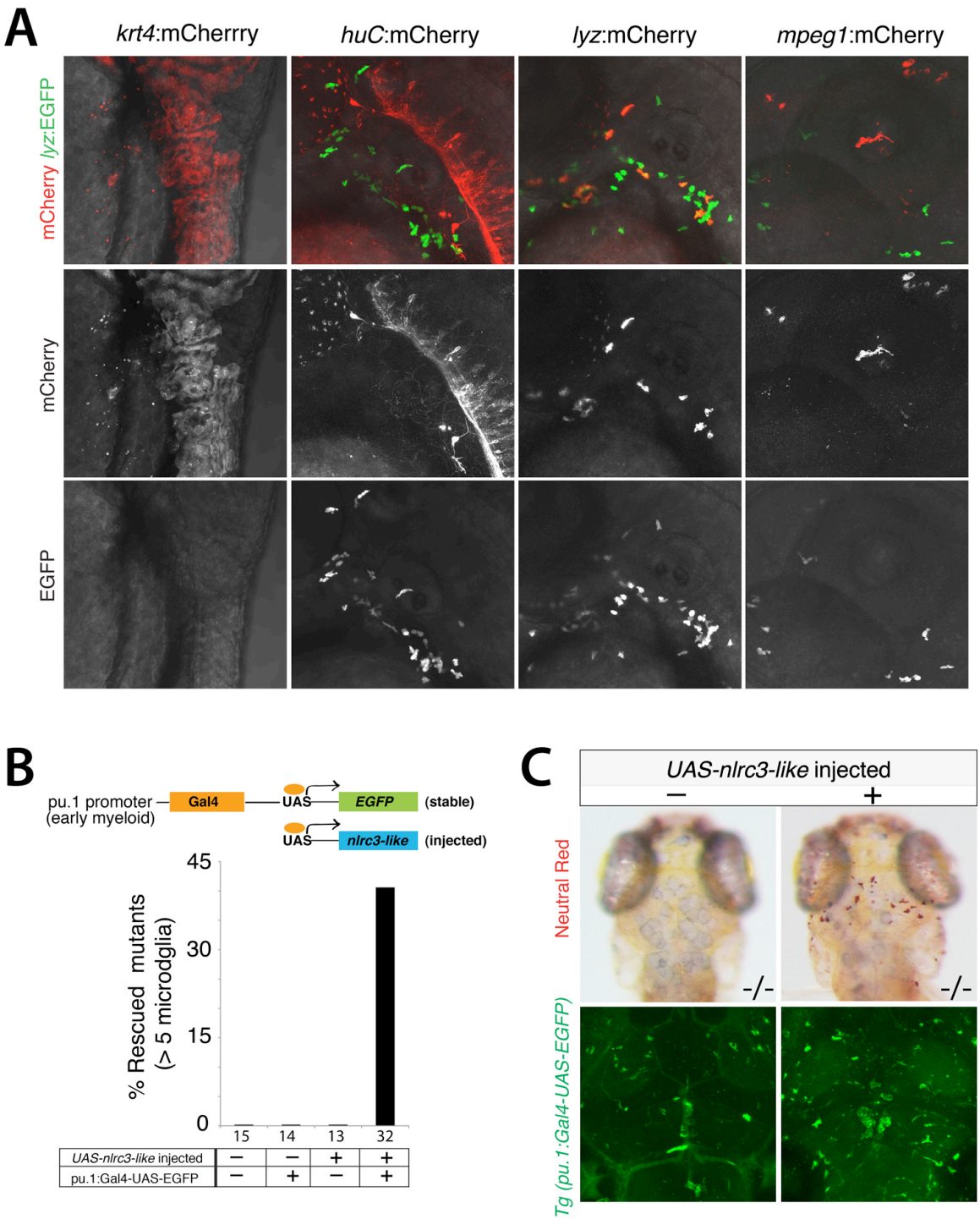
A
krt4:mCherrry
huC:mCherry
lyz:mCherry
mpeg1:mCherry
mCherry lyz:EGFP
mCherry
EGFP
B
pu.1 promoter (early myeloid)
Gal4
UAS
EGFP
(stable)
UAS
nlrc3-like
(injected)
% Rescued mutants (> 5 microdglia)
45
30
15
0
15
14
13
32
UAS-nlrc3-like injected
pu.1:Gal4-UAS-EGFP
C
UAS-nlrc3-like injected
Neutral Red
Tg (pu.1:Gal4-UAS-EGFP)
-/-
-/-

Figure S4. Tissue-specific promoter constructs drive expression in a tissue restricted manner; restoring *nlrc3-like* expression using the myeloid *pu.1* driver rescues microglia in *nlrc3-like*$^{-/-}$ mutants, Related to Figure 5.

(**A**) Top row, merged images showing *lyz:EGFP* stable transgene expression that marks neutrophils and control mCherry expression under the control of different tissue promoters as indicated at 2 dpf. Neutrophil reporter *lyz:EGFP* provides a reference to indicate the locations where leukocytes normally reside, and to distinguish the neutrophil *lyz* promoter from the macrophage *mpeg1* driver. Top row, merged images of mCherry and EGFP expression; middle row, mCherry expression only from the construct injected; bottom row, stable neurtrophil transgene *lyz:EGFP* expression only. (**B**) Schematic showing the Gal4/UAS system applied to drive *nlrc3-like* in early myeloid cells under the control of the *pu.1* promoter. Bar graph showing that 41% of mutants having both the *pu.1: Gal4-UAS-EGFP* driver and *UAS-nlrc3-like* construct are rescued, with > 5 microglia. There was no rescue in mutants missing at least one component of the Gal4/UAS system for *nlrc3-like* expression. (**C**) Representative images showing rescue of a 4 dpf mutant using the Gal4/UAS system (right). Top row, neutral red staining to visualize microglia, and bottom row, images showing *UAS-GFP* expression in the same larvae as above, indicating robust activity of the *pu.1:Gal4* driver. Number below bar graph represents n, number of mutants analyzed.

Figure S5:

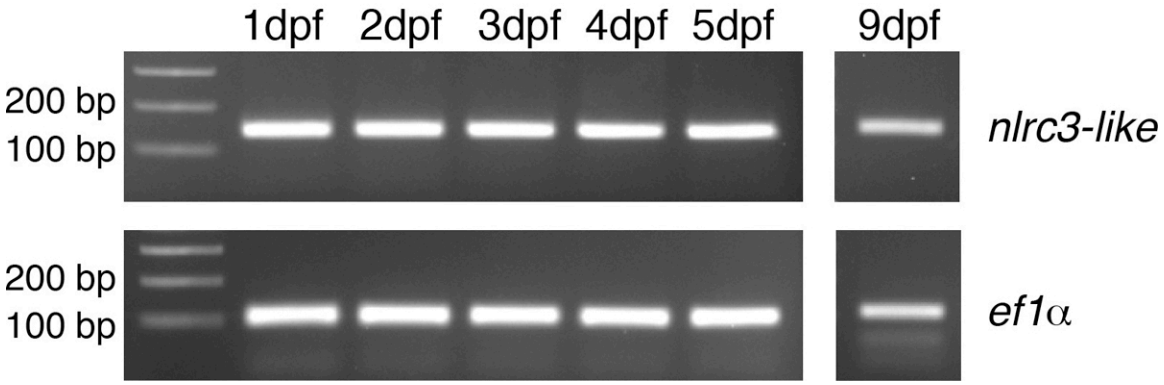

Figure S5. *nlrc3-like* mRNA is expressed throughout embryogenesis and larval development from 1 dpf to 9 dpf, Related to Figure 5. RT-PCR of pooled embryos at 1–5 dpf and individual larvae at 9 dpf show expression of *nlrc3-like* and, as a control, *ef1α*.

Table S1:

A **Primers for qPCR or RT-PCR**

	ef1a
F	AGGACATCCGTCGTGGTAAT
R	AGAGATCTGACCAGGGTGGTT
	b-actin
F	GAGATGATGCCCCTCGTG
R	CTGAGCCTCATCTCCCACAT
	il-1b
F	GCCTGTGTGTTTGGGAATCT
R	TGATAAACCAACCGGGACA
	il-8
F	AGCTTGAG GGTCTGGCTGTAGA
R	GCGTCGGCTTTCTGTTTCA
	il-12a
F	AGCAGGACTTGTTTGCTGGT
R	TCCACTGCGCTGAAGTTAGA
	tnfa
F	GCGCTTTTCTGAATCCTACG
R	TGCCCAGTCTGTCTCCTTCT
	il-10
F	ATTTGTGGAGGGCTTTCCTT
R	AGAGCTGTTGGCAGAATGGT
	nlrc3-like
F	CAACATACACACACCGCCTT
R	TCGTGGCGTAGTTCTTCTTG

B **Primers for Gateway cloning**

	huC promoter
F	ggggacaactttgtatagaaaagttgctGAATTCACTAATTTGAATTTA
R	ggggactgctttttttgtacaaacttgcTCTTGACGTACAAAGATGATA
	krt4 promoter
F	ggggacaactttgtatagaaaagttgctCCTTCCCTTCTACTTTTGACGTCC
R	ggggactgctttttttgtacaaacttgcCCGGATCCTGTGTCTTTGAGTTGC
	lyz promoter
F	ggggacaactttgtatagaaaagttgctGCCATACATTTGATTAGAAG
R	ggggactgctttttttgtacaaacttgcTGTATCACTGCTGATATCTG
	mpeg1 promoter
F	ggggacaactttgtatagaaaagttgctTTGGAGCACATCTGACATC
R	ggggactgctttttttgtacaaacttgcTTGCTGTCTCCTGCACTAATGTG
	mCherry-CAAX
F	GCCGCCACCATGGTGAGCAAGGGCGAG
R	TCAGGAGAGCACACACTT
	nlrc3-like
F	GCCACCATGAGCAATTTTGCTGAAG
R	TTCTCCTCATCTCCTGACCTC

Table S1. Primers used for qPCR, RT-PCR, and Gateway multi-site cloning, Related to Experimental Procedures.

(**A**) Primers used for the qPCR or RT-PCR experiments to examine mRNA levels of various genes are listed. Primer sequences for *il-1β* (Lopez-Munoz et al., 2009), *il-8* (Oehlers et al., 2010), *il-12a* (Lopez-Munoz et al., 2009), *tnfα* (Lopez-Munoz et al., 2009), and *il-10* (Lopez-Munoz et al., 2009) have been previously published. (**B**) Primer sequences used to isolate different gene promoters, and coding sequences of *mCherry-CAAX* and *nlrc3-like* are shown. Lower case indicates the adaptor sequences required for Gateway cloning.