Supplemental Movie Legends

Movie S1. Time-lapse imaging of wildtype macrophages on the yolk sac, Related to Figure 2.

Yolk sac macrophages of control heterozygous *nlrc3-like*$^{+/-}$ embryo carrying the *mpeg1:EGFP* transgene were imaged at 2 dpf. Z-stack images were taken from a ventrolateral view of the yolk at 20-second intervals. *mpeg1:EGFP+* macrophages were typically dispersed in different places on the yolk sac without any apparent region of target, direction, or concentration. In the left panel, cell migration paths of tracked cells are shown. 18 minutes in real time; 20x; 7 fps.

Movie S2. Time-lapse imaging of *nlrc3-like*$^{-/-}$ mutant yolk sac macrophages showing aberrant recruitment of macrophages into large clusters, Related to Figure 2.

nlrc3-like$^{-/-}$ mutant yolk sac macrophages were imaged at 2 dpf in the same conditions as in Movie S1. In contrast to wildtype, macrophages in the mutant form large aggregates, which grow as macrophages join the cluster. Cell tracking (left panel) shows active recruitment of macrophages into the aberrant aggregates. 36 minutes in real time; 20x; 7 fps.

Movie S3. Time-lapse imaging of macrophage migration into the brain in the intact heterozygous *nlrc3-like*$^{+/-}$ embryo at 2 dpf, Related to Figure 2.

Macrophage reporter *mpeg1:EGFP* construct was transiently expressed in an embryo carrying a stable *kdrl:mCherry-CAAX* transgene, which marks the vasculature. Z-stack images were taken from a lateral view of the head at 20-second intervals. A macrophage that had transitioned into a microglial cell and taken up residence in the brain is evident at the start the movie (yellow arrowhead), while two other macrophages (white arrowheads) are beginning to migrate into the brain from the mid cerebral vessel (mcv). 27 minutes in real time; 20x; 7 fps; MB, midbrain; HB, hindbrain; mcv, mid-cerebral vessel; phc, primordial hindbrain channel.

Movie S4. Time-lapse imaging reveals macrophage migration defects in *nlrc3-like*$^{-/-}$ mutant embryo at 2 dpf, Related to Figure 2.

A homozygous mutant embryo carrying the same transgenes as in Movie S3 was imaged under the same conditions. In the *nlrc3-like*$^{-/-}$ mutant, macrophages were found in the head and were motile, but they remained associated with vasculature (arrowheads) and did not take up residence in the brain. 27 minutes in real time; 20x; 7 fps; MB, midbrain; HB, hindbrain; mcv, mid-cerebral vessel; phc, primordial hindbrain channel.

Movie S5. Time-lapse imaging of neutrophils in the head of control *nlrc3-like*$^{+/+}$ sibling at 2.5 dpf, Related to Figure 4.

Consistent with static analysis, dynamic imaging of wildtype embryos showed that neutrophils remained in the periphery and did not enter the brain. Imaging was conducted in double transgenic embryos carrying the *lyz:EGFP* and *kdrl:mCherry-CAAX* transgenes, which mark neutrophils and vasculature, respectively. Dorsal view of the head, with images collected at 20-second intervals. Overlays of GFP (green), mCherry (red), and bright field (blue) images are shown. 20 minutes in real time; 20x; 7 fps.

Movie S6. Time-lapse imaging shows abnormal infiltration of neutrophils into the brain of *nlrc3-like*$^{-/-}$ mutant at 2.5 dpf, Related to figure 4.

Time-lapse imagies of neutrophils in the mutant were captured in the same region and conditions as in Movie S5. In the mutant, neutrophils roamed through the brain, moving in and out of brain vessels. Overlays of GFP (green), mCherry (red), and bright field (blue) images are shown. 20 minutes in real time; 20x; 7 fps.

Movie S7. Time-lapse imaging of neutrophils in heterozygous *nlrc3-like*$^{+/-}$ embryo at 1.5 dpf, Related to Figure 4.

Time-lapse images were taken of *lyz:EGFP* transgenic embryos at 20-seconds intervals. Overlays of GFP (green) and bright field images are shown. Neutrophils remained in the periphery, occasionally entering into circulation, but not into the brain. 34 minutes in real time; 10x; 7 fps.

Movie S8. Time-lapse imaging of neutrophils in *nlrc3-like*$^{-/-}$ mutant embryo at 1.5 dpf shows abnormally high numbers in circulation as well as aberrant brain infiltration, Related to Figure 4.

Neutrophils in live intact *nlrc3-like* mutant embryos were imaged in the same conditions as in Movie S7. Time-lapse movie shows many neutrophils circulating in the vasculature as well as several moving inside the brain. 34 minutes in real time; 10x; 7 fps.

Extended Experimental Procedures

Time-lapse and fluorescent imaging

Maximum intensity projections of z-stacks were made and analyzed using ImageJ or Zeiss LSM software, and processed with Adobe Photoshop CS5. Time-lapse imaging was captured at 1 z-stack per 20 seconds using the 20x objective, except 10x objective was used for imaging neutrophils in circulation. For neutrophil circulation analysis, time-lapse imaging was conducted over a total time of ~ 20 – 120 minutes per embryo analyzed, and the number of neutrophils passing through the duct of Cuvier (yolk sac circulation valley) was calculated from the total number of moving *lyz:EGFP*+ neutrophils seen in a lateral view of the embryo over the total time of the imaging. Number of neutrophils in brain per embryo analyzed was approximated from the number of *lyz:EGFP*+ cells in the midbrain region of the embryonic head where brain macrophages are generally located, albeit including cells that may be in the cranial vasculature, from static and time-lapse images. Statistical significance for the neutrophil analyses was determined by the Student's t-test and with Welch's correction if the variances are significantly different based on the F-test. Percentage of macrophages exhibiting vacuolation (≥ 1 large vacuole) or cell death (TUNEL staining overlapping macrophage nucleus (*mpeg1:EGFP*+/DAPI+) or *mpeg1:EGFP* macrophage debris) was determined from the field of view using the 63x objective of the ventrolateral side of the yolk in 2.5 dpf embryos. Cell tracking of yolk sac macrophages was conducted using the MTrackJ program(Meijering et al., 2012) in ImageJ.

Expression constructs

To construct UAS-nlrc3-like, the full-length *nlrc3-like* sequence was cloned into a Tol2-pBH-UAS vector using the CloneEZ kit (GenScript). The vector to express *mpeg1:EGFP* transiently was made by amplifying the *mpeg1* promoter and regulatory sequences using primers previously published(Ellett et al., 2011), then inserting the *mpeg1* sequence into the pT2AL200R150G plasmid (Urasaki et al., 2006) to replace the *ef1a* promoter. Tissue-specific expression vectors were

assembled using multisite Gateway methods (Kwan et al., 2007). Tissue-specific regulatory sequences used to drive expression in skin (*keratin 4/krt4*)(Chen et al., 2011), neurons (*elavl3/huC*)(Park et al., 2000), neutrophils (*lyz*)(Kitaguchi et al., 2009), or macrophages (*mpeg1*)(Ellett et al., 2011) were amplified from high quality genomic DNA purified from wildtype TL adult fin or muscle. Full-length coding sequence of *nlrc3-like* or control mCherry was inserted downstream of the promoter sequence. Primers used in cloning are listed in Table S1.

RNA extraction, RT-PCR, and quantitative PCR (qPCR)

For primer sequences used, see Table S1. Relative levels of gene expression were measured using the standard curve method as described in the Applied Biosystems manual. Each biological sample (*n*) was run in triplicate and average C_T (threshold cycle) was taken from at least two C_T values with ≤ 0.3 difference. Amount of target gene expression per sample was normalized to its amount of reference *ef1α* expression; expression of *β-actin* was also used as control. Fold difference in target gene was calculated from the average of normalized target gene expression in mutants relative to the average of the control individuals. Each *n* represents an individual embryo measured. Statistical significance was determined by the Mann-Whitney test or Student's t-test.

Protein interaction pull down assay

Cells were lysed in ice-cold lysis buffer (10 mM HEPES (pH7.4), 142.4 mM KCl, 5 mM MgCl2, 1 mM EDTA, 0.2% triton, 1 mg/ml bovine serum albumin, 1 mM dithiothreitol) supplemented with protease inhibitors (Complete cocktail, Roche) using a series of short pulses of sonication (fives times 5 seconds with 10-seconds off intervals). 50 μl of either gluthatione agarose or amylose resin beads were washed three times and incubated with either the GST- or MBP-tagged bait protein lysate, respectively, at 4 °C for 1 hour and then washed extensively. The prey protein lysate was incubated with the bait bound beads in binding buffer (10 mM HEPES (pH7.4), 142.4 mM KCl, 5 mM MgCl2, 1 mM EDTA, 0.2% triton, 1 mg/ml bovine serum albumin, 1 mM dithiothreitol, and protease inhibitors) overnight at

4 °C. Bound proteins were washed extensively in wash buffer (10 mM HEPES, 800 mM NaCl, 0.5% triton) supplemented with protease inhibitors, and analyzed by SDS-PAGE, immunoblotted with anti-GST (clone GST-2, Sigma) or anti-MBP (ab9084, Abcam) antibodies and HRP-conjugated secondary antibodies, and detected using the standard ECL reagent (Pierce).

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