## Supplemental Information



Supplemental Figure 1. **Cytological analysis of embryonic myeloid cell development.** *A-F*, Giemsa staining shows the typical morphology of GFP<sup>+</sup> cells isolated from 20 hpf (*A*), 24 hpf (*B-C*), 48 hpf (*D-E*) and 72 hpf (*F*) Tg(coro1a:eGFP) embryos. *A* indicates the myeloid progenitors at 20 hpf. *B-C* represents young (*B*) and functional (*C*) monocytes/macrophages characterized by high cytoplasmic-to-nuclear ratios, bubbled contour (red arrowhead), and agranular cytoplasm containing various sizes of vacuoles (blue arrows). Blue arrowhead in *C* indicates the ingested debris inside a monocyte/macrophage. *D* shows a neutrophil with the typical bilobe nucleus. *E* reveals a typical matured monocyte/macrophage with vacuoles (blue arrow) and weak basophilic cytoplasm staining. *F* represents a typical dendritic-like cell. The Red arrow in *F* indicates the dendritic filaments. Scar bar: 1 µm. *G*, Histogram reveals the percentages of different cell types at each stage (n ≥ 300).



Supplemental Figure 2. The behavior of apoptotic neutrophils and morphological alteration of macrophages post amputation. *A*, Frames (60X, DIC image) of different time points show an apoptotic neutrophil after internalizing cell debris. Blue and red arrows represent the granules and the nuclei respectively; whereas black and green arrowheads indicate the cell debris ingested and the protrusion bubble of neutrophil membrane respectively. Scar bar: 1  $\mu$ m. *B-E*, Images indicate a bean-like morphology of the GFP<sup>+</sup> macrophages in the resolution stage (*B* and *C*, n = 13) and a long and thin shape in the remodeling/regeneration stage (*D-E*, n = 13). *C* and *E* are 40X magnitude images. Macrophages (green color) in *C* and *E* are indicated by red arrows. Scar bar: 10  $\mu$ m. *F*, Histogram reveals that the length of macrophages in remodeling/regeneration stage is nuch longer than those in inflammation stage (n = 13, mean±SE). Asterisk in *F* indicates statistical differences with corresponding control (t-test, P < 0.05).



Supplemental Figure 3. Neutrophil behavior in *irf8* MO after fin amputation. *A*, Frames of different time points following tail transaction in the 72 hpf *irf8* Tg(coro1a:eGFP; lyz:Dsred) morphants (n = 7). Macrophages are labeled with green fluorescence, whereas neutrophils are labeled with yellow fluorescence. Scar bar: 100 µm. *B*, Histogram reveals that the velocity of neutrophils does not show obvious difference between control and the *irf8* Tg(coro1a:eGFP; lyz:Dsred) morphants (n ≥ 7, mean±SE). *C*, Image reveals that the majority of myeloid cells in the 3 dpf *irf8* Tg(coro1a:eGFP; lyz:Dsred) morphants (n ≥ 7, mean±SE). *C*, Image reveals that the majority of myeloid cells in the 3 dpf *irf8* Tg(coro1a:eGFP; lyz:Dsred) morphants (n = 15) are neutrophils. Scar bar: 100 µm. The white arrows in *C* indicate yellow neutrophils, whereas the red arrow in *C* represents a GFP<sup>+</sup> macrophage. *D*-*F*, Fluorescent imaging (40X) reveals that  $lyz^+$  neutrophils (*D*) can engulf AO<sup>+</sup> (*E*) and AO<sup>-</sup> cell pieces at 2 hpa (n = 11). *F*, Merged view of *D* and *E* with DIC image. Scar bar: 5 µm. White arrowheads and blue arrows in *F* indicate the AO<sup>+</sup> cell particles and AO<sup>-</sup> vacuolar cell pieces engulfed respectively.

Supplemental Movie 1. Behaviors and moving trajectory of neutrophils (yellow) and macrophages (green) during the first 2.5 hpa in the 72 hpf Tg(corola:eGFP; lyz:Dsred) embryos. Neutrophils (yellow) migrate with a faster speed than macrophages (green).

Supplemental Movie 2. Live imaging indicates a neutrophil undergoes apoptosis after internalizing cell debris under VE-DIC microscopic analysis. Notably, the appearance of the neutrophil changes from amoeboid to round shape and finally undergoes a series of morphological alterations that resemble the characteristics of apoptosis including membrane blebbing, chromatin condensation, and nuclear fragmentation

Supplemental Movie 3. Behaviors and moving trajectory of neutrophils (yellow) and macrophages (green) during the first 2.5 hpa in the 72 hpf *irf8* Tg(coro1a:eGFP; lyz:Dsred) morphants. Majority of the cells are neutrophils (yellow) in the *irf8* MO morphants.