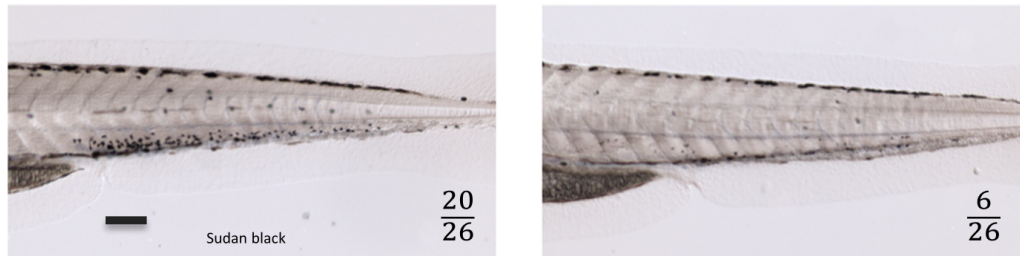
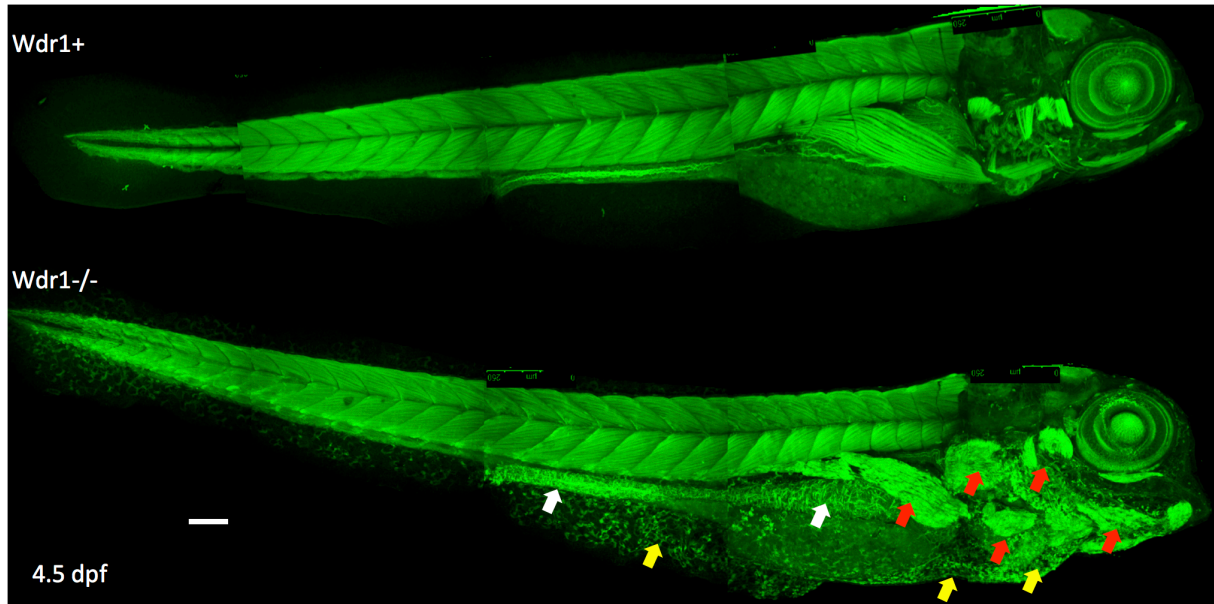


carmin fails to complement  $wdr^{hi1532aTg}$



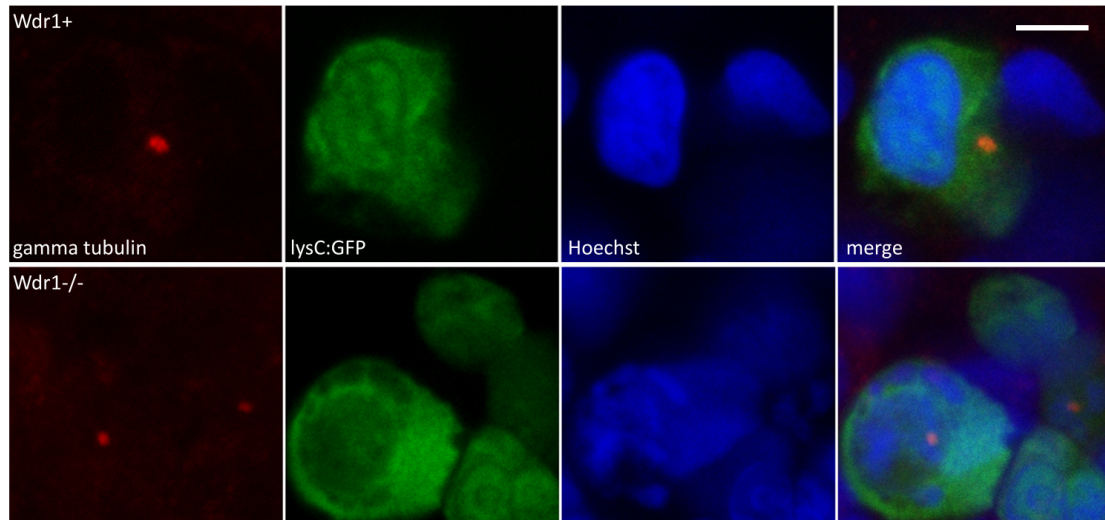
**Figure S1. A *wdr1* retroviral insertion mutant does not complement *carmin*.**

Sperm from the Boston mutant hi1532 (Amsterdam et al., 2004) was used to fertilize eggs from a *carmin* allele carrier female. The offspring was raised for 4 days; 26 larvae were fixed and stained with Sudan black to reveal neutrophils. Scale bar, 100  $\mu$ m



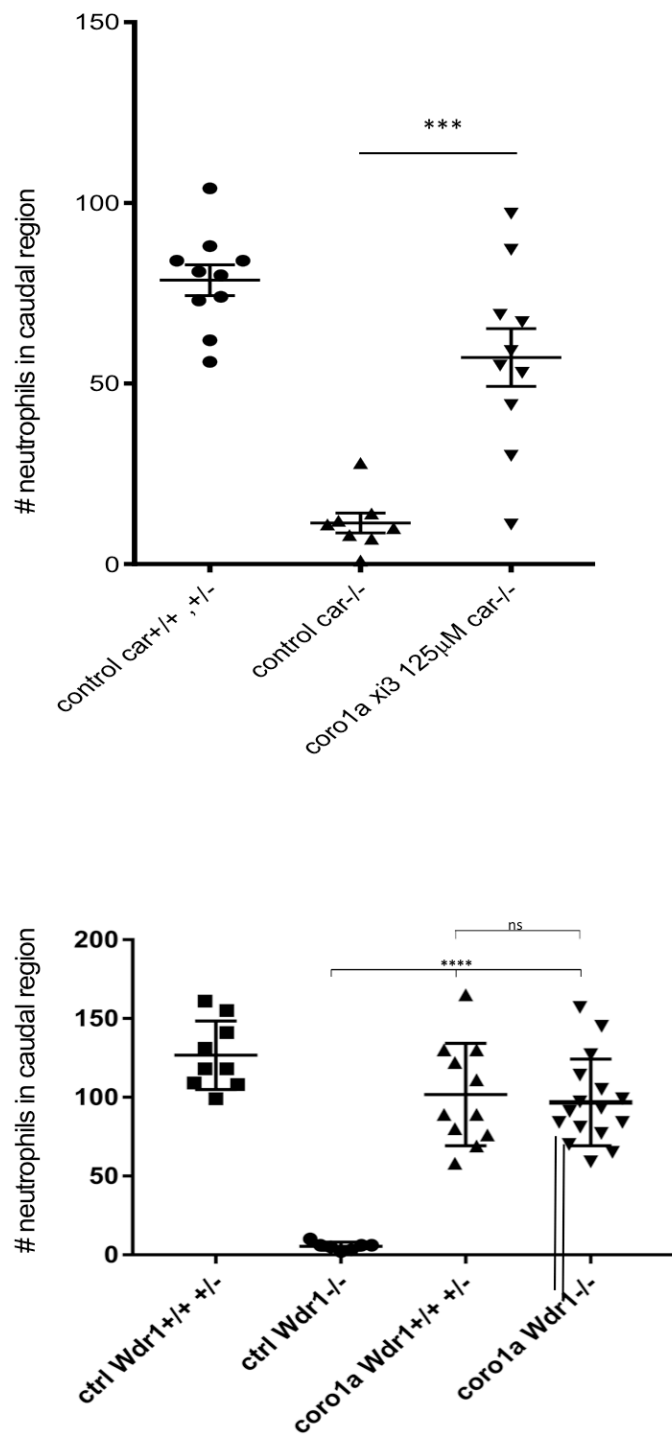
**Figure S2. Developmental defects of *carmin* mutant larvae**

Phalloidin labelling of F-actin at 4.5 dpf. Arrows indicate the accumulation of F-actin in the cortex of epidermal cells (yellow), in the gut (white), and the malformation of head muscles (red) in the mutant larvae. Maximum projections from 5 adjacent confocal z-stacks were stitched together to cover the entire larva. Scale bar, 100  $\mu$ m



**Figure S3. Gamma tubulin immunodetection in neutrophils reveals only 1 centrosome per mutant neutrophil, excluding aneuploidy.**

Tg(Lyz:GFP) embryos obtained from *carmin* carrier parents were immunostained at 62 hpf for gamma-tubulin (red), stained with Hoechst 33342, and examined by confocal imaging. All imaged mutant (n=12) and WT (=10) neutrophils showed one and only one gamma-tubulin stained centrosome. Scale bar, 10  $\mu$ m.



**Figure S4.** A splice blocking morpholino, *coro1a-xi3*, rescues *Wdr1*-deficient neutrophils (upper panel; n=10 control and 18 *coro1a-xi3* injected embryos, all from the same experiment) as does the translation blocking morpholino *coro1a* ATG (lower panel; n=18 control and 27 *coro1a*-ATG injected embryos, all from the same experiment).

## Video Legends

**Video 1 (related to Fig. 1).** Timelapse confocal imaging of Tg(lyz:GFP) *carmin* mutant and sibling embryos from 56 hpf reveals the gradual decrease in neutrophil number and their reduced motility in the mutant (duration 16 hrs, 37min; time interval 19 min, 11 sec).

**Video 2 (related to Fig. 2).** Timelapse confocal imaging (14 hrs) starting at 54 hpf of Tg(mpx-GFP) WT sibling (left) and *carmin* mutant (right) embryos injected with an mpx:Lifect-ruby plasmid reveal that whereas F-actin in sibling neutrophils is enriched at the cell cortex and at transient cell constrictions associated with their ameoboid motility, mutant neutrophils have large clumps of F-actin that remodelled only slowly. Time is indicated in hrs:min:sec. Representative of 15 time-lapse imaging sessions.

**Video 3 (related to Fig. 3).** A confocal Z-stack through a mutant neutrophil nucleus showing that mutant nuclei become multiglobular (n=12).

**Video 4 (related to Fig. 3).** Timelapse confocal imaging of *carmin* Tg(mpx:GFP) embryos transiently expressing lyz:H2b-mCherry; WT sibling (left) and mutant (right). The first image (t=0) shows the bright field image overlaid with the green and red fluorescence. Time is indicated in hrs:min.

**Video 5 (related to Fig. 3).** Timelapse confocal imaging of *carmin* Tg(mpx:GFP) embryos transiently expressing lyz:H2b-mCherry reveals a striking, continuous unwinding of the mCherry+ chromatin of GFP+ neutrophils, after which the chromatin rapidly condenses in separate masses (by t=8 hrs for the lower neutrophil), and the cell erupts (by t=11 hrs). Time is indicated in hrs:min:sec (time interval, 3 min 20 sec.). Representative of 20 time-lapse confocal imaging sessions of 15 hrs.

**Video 6 (related to Fig. 3).** Timelapse imaging (15 hrs; time interval 2min50) of Tg(mpx:GFP) *carmin* mutant embryo transiently expressing lyz:H2b-mCherry. Even though macrophages are not labelled here by expression of a fluorescent reporter, their motion can be seen due to the H2b-mCherry protein of neutrophils that they previously phagocytosed (the GFP of engulfed neutrophils is no longer seen, as it loses its fluorescence in the acidic phagolysosome compartment of the macrophage). Such macrophages wandering near still alive mutant neutrophils do not engulf them while the latter's chromatin unwinds for hours; they only approach and engulf them after the neutrophils have reached the final stages of DNA condensation and/or cell eruption.

**Video 7 (related to Fig. 4).** A z-stack through the lamin B2 and DAPI-stained nucleus shows that dissolution of the nuclear lamina can occur prior to the late stage of GFP exclusion from the condensed chromatin. Arrows indicate three neutrophils in varied stages of nuclear herniation/chromatin unwinding, that show no lamin B2 signal, and no GFP depletion of the nuclear material. Surrounding cells all have their nucleus well delineated by lamin B2.

**Video 8 (related to Fig. 5).** Time-lapse confocal imaging of Tg(mpx:GFP; lyz:H2b-mCherry) embryos after 12 hrs incubation with 100  $\mu$ M Rockout (real-time duration 4h:43min; time interval 4min:38s).

**Video 9 (related to Fig. 7).** Coronin 1A morpholino-injected embryos were mounted in agarose and time-lapse imaged in parallel via a wide-field fluorescence microscope for 20 hrs,

starting at 28 hpf, with a time-lapse interval of 6 min. While untreated *Wdr1*<sup>-/-</sup> mutants displayed gradual neutrophil disappearance, morpholino-treated *Wdr1*<sup>-/-</sup> embryos showed a steady expansion of neutrophil number and neutrophil motility similar to WT with or without corola Mo.