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Title: Coronin 1A depletion restores the nuclear stability and viability of Aip1/Wdr1 deficient neutrophils

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Running title : actomyosin contractility maintains neutrophil nuclear integrity

eTOC summary:

Bowes et al. show that in zebrafish embryos deficient in the cofilin cofactor AIP1/Wdr1, neutrophils display F-actin as cytoplasmic aggregates, spatially uncoupled from active myosin, then a progressive unwinding of their nucleus, followed by eruptive cell death. This adverse phenotype is fully rescued by depletion of another cofilin cofactor, coronin 1A.

ABSTRACT

Actin dynamics is central for cells, and especially for the fast moving leukocytes. The severing of actin filaments is mainly achieved by cofilin, assisted by Aip1/Wdr1 and coronins. We found that in Wdr1-deficient zebrafish embryos, neutrophils display F-actin cytoplasmic aggregates, and a complete spatial uncoupling of phospho-myosin from F-actin. They then undergo an unprecedented gradual disorganisation of their nucleus, followed by eruptive cell death. Their cofilin is mostly unphosphorylated and associated with F-actin, thus likely outcompeting myosin for F-actin binding. Myosin inhibition reproduces in wild-type embryos the nuclear instability and eruptive death of neutrophils seen in Wdr1-deficient embryos. Strikingly, depletion of the main coronin of leukocytes, coronin 1A, fully restores the cortical location of F-actin, nuclear integrity, viability and mobility of Wdr1-deficient neutrophils *in vivo*. Our study points to an essential role of actomyosin contractility in maintaining the integrity of the nucleus of neutrophils, and a new twist in the interplay of cofilin, Wdr1 and coronin in regulating F-actin dynamics.

INTRODUCTION

The turnover of actin filaments (F-actin) is essential for the highly mobile leukocytes. As F-actin is a stable polymer, its turnover requires molecular machineries to polymerise and depolymerise or sever actin filaments in a spatially and temporally coordinated manner within the cell. Several protein complexes driving F-actin polymerisation in colinear or variously branched configurations have been identified (Blanchoin et al., 2014). F-actin severing is mainly accomplished by small proteins, the cofilins, with the help of facilitating co-factors, the best known of which are Aip1 (Actin Interacting Protein 1, also called Wdr1 due to its primary and tertiary structure (Ono, 2017)), and the coronins (Bamburg et al., 1999;

Andrianantoandro and Pollard, 2006; Briehar, 2013; Cai et al., 2007; Gandhi and Goode, 2008; Gressin et al., 2015). Cofilins bind somewhat cooperatively to F-actin, and F-actin severing by cofilin alone occurs at boundaries between cofilin-covered and bare F-actin. F-actin filaments fully covered by cofilin lack such boundaries, and are therefore stabilized rather than severed (Andrianantoandro and Pollard, 2006). Wdr1 is able to bind cofilin-loaded F-actin and induce their rapid severing (Nadkarni and Briehar, 2014). Recent studies have shown that, among other modes of action (Cai et al., 2007; Galkin et al., 2001; Gandhi and Goode, 2008), coronin binding to F-actin facilitates the further binding of cofilin and subsequent F-actin severing (Jansen et al., 2015). However, since most of these results are from experiments using in vitro assays, the extent to which they represent actin dynamics in the highly mobile leukocytes remains to be assessed. Cofilins, coronins and Wdr1 are highly expressed in neutrophils (Fagerberg et al., 2014; Singh et al., 2013b). Among leukocytes, neutrophils appear to be especially sensitive to perturbations of their actin dynamics, which can even compromise their viability. For example, X-linked congenital neutropenia in humans is due to an activating mutation in WASP, a protein fostering F-actin polymerization expressed in all leukocytes. Yet this mutation, which expectedly causes F-actin excess in all leukocytes, is lethal only for the neutrophils (Moulding et al., 2007, 2012). Other inherited human immunodeficiencies have been described that are mainly due to defective neutrophil migration to infection sites. Such is the case for several 'neutrophil cytoskeletal diseases' (Nunoi et al., 2001), including the 'Lazy Leukocyte Syndrome' (Goldman et al., 1984; Miller et al., 1971), in which the mainly cortical (i.e. sub-plasmalemmal) location of F-actin is replaced by F-actin aggregates in the cytoplasm. Several LLS cases were recently shown to be due to mutations in WDR1 (Kuhns et al., 2016; Pfajfer et al., 2018). However, these cytoskeletal defects are difficult to study since human (or mouse) neutrophils are very short-lived in cell culture.

Due to its transparency and very small size, the developing zebrafish has become a very attractive vertebrate model to study the biology of neutrophils in vivo, down to sub-cellular resolution (Le Guyader et al., 2008; Sarris et al., 2012; Shelef et al., 2013; Yoo et al., 2010, 2012). In a forward genetic screen for neutropenic zebrafish, we isolated a *Wdr1*-deficient mutant, in which neutrophils are initially produced in the embryo, but then quickly decline and disappear. We found that beyond their perturbed actin dynamics and motility, neutrophils undergo an unexpected disintegration of their nuclear envelope, preceding their death. This nuclear phenotype is phenocopied in wild-type embryos by a mere inhibition of actomyosin contractility. Conversely, the deleterious effects of *Wdr1* deficiency on the neutrophils are entirely rescued by depletion of their major coronin, coronin 1A. Our results altogether uncover vital interactions between cofilin, *Wdr1*, coronin, and actomyosin contractility in neutrophils in vivo. They also open a new prospect for the future treatment of LLS patients.

RESULTS

***Wdr1* is essential for the maintenance of neutrophils in developing zebrafish**

An N-ethyl-N-nitrosourea mutagenesis screen for recessive mutations causing neutropenia in zebrafish swimming larvae led to the identification of a mutant, which we called *carmin*, that had no neutrophils while displaying otherwise normal overall morphology (Fig. 1 A). High resolution positional mapping identified the mutated gene as *wdr1*. Corroborating this, a *wdr1* retroviral insertion mutant from the Hopkins collection (Amsterdam et al., 2004) failed to genetically complement *carmin* (Fig. S1), and two morpholinos designed against *wdr1* RNA reproduced the *carmin* phenotype in wild-type (WT) embryos (Fig. 1 D), indicating that the *carmin* allele acts as a *Wdr1* deficiency. The *wdr1* gene encodes a protein containing 14 WD40 repeats that fold into two β -propeller domains, each consisting of seven ‘blades’ (Ono,

2017; Voegtli et al., 2003). The *carmin* allele comprises a T to C transversion causing the substitution of a tryptophan residue conserved from yeast to man into an arginine in the C-terminal WD40 repeat of the protein (Fig. 1 C).

We transferred the *carmin* allele into transgenic backgrounds highlighting neutrophils (Tg(mpx:GFP), Tg(lyz:GFP) or Tg(lyz:DsRed) so as to track the impact of Wdr1 deficiency on neutrophil fate in live developing embryos. Neutrophil counts following confocal microscopy of live embryos over time confirmed that both *carmin* embryos and their siblings were initially able to effectively produce neutrophils, but their number then steadily declined, such that by 72 hpf *carmin* larvae had less than 10% of the wild-type neutrophil population (Fig. 1 B; and Video 1).

Wdr1 deficiency causes systemic aggregation of actin, and nuclear instability in neutrophils

Wdr1 / Aip1 is known as a co-factor of cofilin, the main effector of F-actin depolymerisation (Ono, 2003, 2017). We therefore combined the detection of neutrophils and of F-actin in mutant and sibling embryos and larvae. Wdr1 deficiency led to the accumulation of F-actin in the *carmin* mutant. These F-actin aggregates were first seen by 52 hpf in neutrophils as intracytoplasmic clumps, and in the caudal fin epidermis as excess cortical actin (Fig. 2 A, B); then over the next days they progressively accumulated throughout the body, notably the epidermis and epithelial tubes (gut, pronephric ducts and tubules) (Fig. S2) with concomitant spreading of edema, until death by 8-10 dpf.

To observe the dynamics of F-actin in the neutrophils of mutant vs. sibling embryos, we performed time-lapse confocal imaging of Tg(mpx:GFP) embryos injected with a plasmid driving expression of the F-actin reporter Lifeact-Ruby in neutrophils (Yoo et al., 2010). *Carmin* neutrophils harboured intense F-actin clumps that remodelled only slowly in contrast

with the neutrophils of sibling embryos that showed the classical enrichment in F-actin at the cell cortex and at transient smaller foci associated with their fast amoeboid motility. (Fig. 2 C; and Video 2).

Whole-mount Hoechst 33342 staining of DNA in fixed embryos revealed that carmin neutrophils seemed to have multiple small abnormal nuclei (Fig. 3 A; and Video 3). However, live confocal imaging of neutrophil nuclei in Tg(mpx:GFP) embryos transiently expressing a Lyz:H2b-mCherry transgene (Yoo et al., 2012) revealed nuclear lobules that were actually joined by unraveled and thinly stretched threads of chromatin (Fig. 3 B; and Video 5). Concordantly, centrosome staining via gamma-tubulin antibody revealed only 1 centrosome per neutrophil examined (Fig. S3) excluding centrosome amplification and aneuploidy. Given that the nuclei of WT zebrafish larval neutrophils, unlike those of humans, are not multilobulated, this indicated that *Wdr1*^{-/-} neutrophils undergo nuclear dysmorphology.

To examine this phenomenon dynamically, we conducted time-lapse confocal imaging of Tg(mpx:GFP) embryos transiently expressing the Lyz:H2b-mCherry transgene to specifically highlight the chromatin of neutrophils (Videos 4, 5, and 6). These time-lapse sequences revealed a striking characteristic, wherein the chromatin of *wdr1*^{-/-} neutrophils appeared to continuously unravel and unwind over the course of 5-10 hours within the intact cell, until it suddenly condensed in discrete clumps within an hour, and the cell then most often erupted into fragments (Fig. 3 B; and Video 5).

The final eruption of carmin neutrophils indicated a cell death mode quite different from classical neutrophil apoptosis seen in mammals and zebrafish (Fox et al., 2010; Loynes et al., 2010), wherein the cell merely shrinks (then optionally inflates, reflecting secondary necrosis) before death. Consistently, only a small proportion of neutrophils (5% by 54 hpf) in live *carmin* embryos had their surface labelled with fluorescent annexin V, indicating that carmin neutrophils for the most part do not die by apoptosis. A greater percentage of neutrophils were

marked by a TUNEL assay (25% at 54 hpf, 37% at 72 hpf), indicative of DNA double-strand breaks, and this always correlated with a more intense staining by Hoechst 33342, indicative of DNA condensation, and with exclusion of GFP from this condensed chromatin (Fig. 3C; n=24). Together with the *in vivo* time-lapse imaging data, this suggests that the neutrophil chromatin is able to unwind extensively for hours within the live cell, until double-strand DNA breaks occur (as detected by Tunel), and trigger chromatin condensation, followed by cell fragmentation.

Concordantly, neighboring and still functional macrophages were not attracted to the neutrophils while their nucleus unwound for hours. They bypassed the neutrophils in this state, and only approached and engulfed them after the latter had passed through the chromatin condensation phase (Video 6). This was compelling evidence that the mutant neutrophils do not secrete or expose death signals at their surface until the ultimate phase of DNA breaks and condensation. Hereafter we will call Late (L) the stage of mutant neutrophil pathology at which chromatin has become condensed, GFP-depleted and Tunel-positive, and Early (E) any stage before that.

The nuclear lamina becomes disrupted in *Wdr1*-deficient neutrophils

We hypothesized that the unraveling of chromatin in mutant neutrophils might be due to disruption of the nuclear envelope (NE). NE disruptions typically occur at gaps in the nuclear lamina, a meshwork of intermediate filament proteins, the lamins, that connect the chromatin to the inner nuclear membrane (Hatch, 2018). As lamin A is known to be virtually absent in neutrophils (at least in mammals; Olins et al., 2008; Rowat et al., 2013), we immuno-detected lamin B2 in whole embryos.

In sibling embryos, while the lamin B immunodetected in neutrophils was lower than in other cell types - in agreement with the literature (Olins et al., 2008), it did delineate the NE (Fig. 4

A). In contrast, about half of the mutant neutrophils displayed no lamin B delineated NE (Fig. 4 B-E), but lamin B aggregates (Fig. 4 D), or no lamin B signal (Fig. 4 B, C). No mutant neutrophil at the L stage showed a Lamin B-delineated NE (n=18; Fig. 4 C, D, F). The lack of lamin B delineated NE was also found in a fraction (2/9) of mutant neutrophils at the E stage (Fig. 4 B; and Video 7).

Perturbation of actomyosin contractility (re)produces chromatin unwinding in neutrophils

How could *Wdr1* deficiency cause the disruption of the nuclear lamina observed in mutant neutrophils? The physiological breakdown of the nuclear lamina during the cell cycle is achieved by phosphorylation of the lamins, notably via the Cyclin-dependent kinase 1 (CDK1) (Ungricht and Kutay, 2017). To test if the mutant nuclear phenotype was due to an inappropriate induction of CDK1, we treated mutant embryos from 2 dpf onwards with a CDK1 inhibitor previously validated in zebrafish embryos, RO-3306 (Strzyz et al., 2015). This treatment did not rescue the mutant neutrophils. Then, since mutant neutrophils displayed abnormal actin organisation and dynamics, we wondered if actomyosin contractility might be perturbed. So we examined the distribution of active myosin vs. F-actin in neutrophils of *carmin* vs. sibling embryos at 2.5 dpf by whole-mount immunodetection of Ser19-phosphorylated myosin light chain (pMLC). This revealed, only in the mutant neutrophils, areas of intense pMLC staining which seemed to ‘cap’ or form thick peripheral bands around the cell (Fig. 5 A, B), in positions quite apart from the F-actin clumps, suggesting an unusual spatial uncoupling of pMLC and F-actin, which would compromise acto-myosin contractility. Interestingly, quantification via 3D image analysis of neutrophils segmented from the tissue by their GFP content showed that mutant neutrophils had pMLC and F-actin mean densities similar to those of WT sibling embryos (Fig. 5 C), indicating that

their prominent F-actin clumps and pMLC caps actually reflected only a local and not a global excess of F-actin and pMLC in the cell.

We examined the spatial uncoupling of F-actin and pMLC more systematically by quantifying their degree of colocalisation within individual neutrophils in mutants vs. sibling embryos (Fig. 5 D). In WT neutrophils, F-actin and pMLC showed a substantial degree of colinear variation (Pearson's coefficient = 0.40) and colocalisation (Manders' coefficient A = 0.63; B = 0.52 – see Figure legend). In sharp contrast, all three coefficients collapsed in the mutant neutrophils; the Pearson coefficient actually collapsed to a mean value of zero (0,0047), and even to a negative value (meaning anti-correlation) for 6/12 cells. Even the two cells in this series that showed the highest (even though still lower than the WT mean) Pearson and Manders correlation displayed a prominent peripheral pMLC cap (Fig. 5B) This analysis thus uncovered a striking uncoupling of pMLC and F-actin localisation throughout the mutant neutrophils, which necessarily precedes the unwinding of the nucleus since we found it in all mutant neutrophils that we examined - either as peripheral pMLC caps and/or as a fine-grained decorrelation of pMLC and F-actin.

Based on these results, we investigated whether pharmacological perturbation of actomyosin contractility in WT neutrophils might reproduce in part or full, the phenotype of *Wdr1* deficient neutrophils. To this aim, we used Rockout, a Rho kinase inhibitor reportedly more efficient in zebrafish embryos than the Y-27632 more widely used for mammalian cells (Weiser and Kimelman, 2012) to reduce actomyosin contractility by inhibiting MLC Ser19 phosphorylation (Maekawa et al., 1999; Wiggan et al., 2012). Incubation of 2 dpf WT Tg(*mpx*:GFP; *Lyz*:H2b-mCherry) embryos with Rockout for 8-16 hours appeared compatible with embryo viability. It reduced the amount of pMLC in neutrophils (Fig. 5 E), and caused a neutrophil phenotype similar to *Wdr1* deficiency, wherein nuclear dysmorphology was

observed including chromatin unraveling, nuclear lamina disruption, and fragmentation / eruption of neutrophils (Fig. 5 F-J; and Video 8).

Cofilin is constitutively active in Wdr1-deficient neutrophils.

What could cause the uncoupling of myosin from F-actin in Wdr1-deficient neutrophils? An attractive candidate was cofilin, with which Wdr1 interacts to help sever and then fully depolymerise F-actin. Myosin and cofilin are indeed known competitors for binding to F-actin (Wiggan et al., 2012; Elam et al., 2013). Cofilin capacity to bind F-actin is tightly regulated by Ser3 phosphorylation – the phosphorylated form (hereafter designated as p-cofilin) is unable to bind F-actin. Therefore, we sought to compare the global amount, localization and phosphorylation state of cofilin in mutant vs. WT neutrophils via whole-mount immunohistochemistry, using antibodies against either all cofilin or p-cofilin.

Both immunostainings demonstrated marked differences between WT and mutant neutrophils. In WT neutrophils, cofilin was fairly evenly distributed throughout the cytoplasm with frequent enrichment at the cell cortex, which was also delineated by F-actin (Fig. 6 A), whereas in mutant neutrophils, cofilin accumulated into dense cytoplasmic areas that appeared to colocalize with the F-actin clumps (Fig. 6 B). The quantitative 3D image analysis revealed that even though the mutants neutrophils had on average one third less total cofilin than the WT (Fig. 6 C), they showed a clearly higher degree of colocalisation of cofilin and F-actin, reflected in the Pearson's as well as Manders' A and B coefficients (Fig. 6 D).

Phospho-cofilin was detected in WT neutrophils throughout the cytoplasm (Fig. 6 E), whereas it was virtually unseen in mutant neutrophils (Fig. 6 F). The quantitative analysis confirmed that the mutant neutrophils contained 4-fold less p-cofilin than the WT (Fig. 6 G). Interestingly, the analysis of p-cofilin / F-actin colocalization (Fig. 6 H) revealed a complete

absence of spatial correlation in the WT neutrophils, with a mean Pearson's coefficient close to zero, consistent with the reported inability of p-cofilin to bind F-actin.

Altogether these data show that in mutant but not WT neutrophils, cofilin is mostly present in non-phosphorylated form, associated with F-actin. It is therefore expected to outcompete myosin for binding to F-actin, and this would lead to the observed spatial uncoupling of F-actin and pMLC. Moreover, the extensive coverage of actin filaments with cofilin in mutant neutrophils is expected to cause F-actin stabilisation rather than severing (Jansen et al., 2015), especially in the absence of Wdr1 which facilitates F-actin severing by cofilin under high cofilin coverage of actin (Nadkarni and Briehner, 2014) (see Discussion). This would lead to the defects in F-actin turnover that we have observed.

Coronin 1a depletion fully rescues Wdr1 deficient neutrophils

Along with Wdr1, coronin 1 is known to influence the rate of F-actin severing by cofilin, either facilitating or hindering it depending on the type of F-actin structure, and other contextual parameters (Briehner, 2013; Briehner et al., 2006; Cai et al., 2007; Chan et al., 2011; Galkin et al., 2008). In addition, coronin 1B has been shown to induce cofilin dephosphorylation via recruitment of Slingshot phosphatase (Cai et al., 2007), so depleting coronin 1 might help normalize the cofilin phosphorylation level in *wdr1^{carmin}* mutants. Coronin 1A is the main coronin in leukocytes. Therefore, we examined the effect of coronin 1A depletion in Wdr1-deficient *carmin* embryos, using antisense morpholinos to inhibit coronin1a mRNA translation or splicing. Quite strikingly, whereas in WT sibling embryos coronin 1A depletion did not appear to affect neutrophil expansion or mobility, in *carmin* embryos it fully rescued neutrophils, restoring not only their numbers through time, but also their actin dynamics, motility, and nuclear morphology (Fig. 7; Fig. S4; and Video 8).

DISCUSSION

Recent studies have demonstrated a critical role of *Wdr1* in the necessary breakdown and turnover of filamentous actin, as reviewed in (Ono, 2017). Moreover, its relevance to human disease has recently been pointed out by reports of *WDR1*-mutated patients that suffered immune deficiencies, of two different types: an autoinflammatory syndrome including neutrophilia, *WDR1* intracellular aggregates and IL18 production (1 family/1mutation (Standing et al., 2017)), and a "Lazy leukocyte syndrome" (LLS), characterized by recurrent infections due to the defective mobilization of neutrophils to inflammatory stimuli (6 families/8 mutations, (Kuhns et al., 2016; Pfajfer et al., 2018)). The phenotype of our *wdr1^{carmin}* mutant zebrafish diverges from the autoinflammatory syndrome, showing no increase in assessed inflammatory cytokines (data not shown), but is close to the LLS. Two typical traits of LLS are excess F-actin in neutrophils, manifesting as F-actin clumps throughout the cytoplasm instead of the normal, mostly cortical localization (Foroozanfar et al., 1984; Goldman et al., 1984), and the herniation of their nuclei (Kuhns et al., 2016). The recent identification of *WDR1* missense mutations in LLS patients from six different families suggests that *WDR1* deficiency could be the cause of most if not all LLS cases. The similar phenotype of neutrophils in our *Wdr1*-deficient zebrafish reinforces that prospect.

Now while in our *wdr1^{carmin}* mutant, neutrophils are the first cell type to be visibly affected during fish development, over the next days the maturation of epithelia and muscles becomes increasingly affected, leading to larval death. In line with this, whole-animal *Wdr1* KO is lethal in mice (at embryonic peri-implantation stage), and *Wdr1* was found important for epithelial polarity, and for sarcomeric actin assembly in striated and cardiac muscles (Ono, 2017; Yuan et al., 2014).

In contrast, it seems that beyond their high rates of infection due to crippled neutrophils, LLS patients developed normally and can live a normal life. In light of our results, we conclude: i) that their WDR1 mutations are probably hypomorphic, unlike the *carmin* mutation, and ii) that the neutrophil is the cell type most sensitive to Wdr1 dysfunction, both in mammals and in zebrafish.

Given the role of Wdr1 / Aip1 in F-actin depolymerization, it is not unexpected that its malfunction first affects the highly mobile neutrophils, as their amoeboid motility requires a high level of F-actin turnover. More enigmatic was the typical herniation of the neutrophil nucleus, seen both in LLS & the auto-inflammatory syndrome. In the zebrafish embryo, thanks to high-magnification in vivo time-lapse imaging, we could see the nuclear herniations develop into a striking "unwinding" of the nuclear material, that last for hours before the cell's demise – a phenomenon so far never reported in any cell type, to our knowledge. We found that this nuclear phenotype is accompanied by a loss of the nuclear lamina, as visualized by lamin B2 immunostaining, that seems to occur early in the "unwinding" process, and is bound to cause nuclear envelope (NE) rupture.

Situations of transient NE rupture and associated nuclear herniations have recently been described in adherent cells, migratory cancer cells, and dendritic cells migrating through constricted spaces, and were always found to occur at the site of gaps in the nuclear lamina (Denais et al., 2016; Hatch, 2018; Hatch and Hetzer, 2016; Lammerding and Wolf, 2016; Raab et al., 2016; Thiam et al., 2016). In all cases, NE rupture was found to originate in mechanical stress, due to e.g. actin stress cables compressing the nucleus or to excess actomyosin contractility (Denais et al., 2016; Hatch, 2018; Hatch and Hetzer, 2016; Lammerding and Wolf, 2016), in which case the LINC complex (that links cytoplasmic F-actin to the nuclear lamina, in a lamin A-dependent way) was often also involved (Khatau et al., 2010; Kim et al., 2017).

However, all these studies dealt with transient and/or local NE ruptures and associated nuclear lamina discontinuities, far from the irreversible nuclear lamina dissolution that we witnessed in *Wdr1*-deficient neutrophils. We think that this extreme phenotype may originate in the special nature of the NE in neutrophils. Mammalian as well as zebrafish neutrophils essentially lack lamin A, have low levels of B-lamins, and lack nesprins, the main component of the LINC complex (Olins et al., 2008, 2009; Singh et al., 2013a). The lamin A level specifies the rigidity of the nuclear envelope; its absence in neutrophils leads to a highly flexible nucleus, which allows them to migrate through smaller pores than any other cell type (Rowat et al., 2013). The downside of lamin A absence is thought to be a more fragile nucleus (Harada et al., 2014; Olins et al., 2009); However this consequence was shown only upon lamin A depletion or mutation in cells that normally express it. The neutrophil nucleus might obey a different logic, whereby its high deformability might actually make it less prone to mechanical stress-induced NE breaks. In the unusual case of the LINC-less neutrophil, (where LINC is thought to be essential for force transmission from the cytoskeleton to the nucleus), the nature of the connection between the nucleus and cytoskeleton remains to be explored. Our data provide a first hint, as we found that in contrast with all cases cited above, where excessive actomyosin contractility favored or triggered NE rupture, actomyosin contractility was necessary to maintain the integrity of the NE of neutrophils in WT zebrafish embryos. We surmise that this also holds for mammalian neutrophils, based on the nuclear phenotype of LLS neutrophils.

Given such a role of actomyosin contractility in neutrophil NE maintenance, our finding that in *carmin* neutrophils, pMLC becomes completely spatially uncoupled from F-actin, predicts the observed breakdown of their NE.

This spatial uncoupling of F-actin and pMLC in *Wdr1*-deficient neutrophils is likely due to the exclusion of myosin from F-actin by competing cofilin (Elam et al., 2013; Kuhn and

Bamburg, 2008; Wiggan et al., 2012). Two phenomena indeed support this hypothesis. Firstly, in the *Wdr1*-deficient neutrophils, unlike in WT, we found cofilin to be mostly in its unphosphorylated, 'active' form (i.e. able to bind F-actin), and consequently most if not all cofilin would be bound to F-actin. This overall dephosphorylation of cofilin was also found in *Wdr1* KO mouse cells (Xiao et al., 2017), and in the neutrophils of an LLS patient harboring a D26N mutation in *Wdr1* (Kuhns et al., 2016). Xiao et al. were able to explain this effect of *Wdr1* deficiency by showing that mouse WDR1 binds to the LIM-Kinase (LIMK1) microtubule binding domain, thus preventing its inhibitory localisation to microtubules and enhancing its ability to phosphorylate cofilin. Interestingly, even though the two point mutations shown to cause cofilin unphosphorylation - our carmin W540R and the human D26N - lie far apart on the *Wdr1* primary sequence, in the 3D crystal structure they both lie near the convex surface of the protein, opposite to the concave surface where cofilin is thought to bind (Ono, 2017). This would suggest that it is the convex side of the *Wdr1* protein that binds the PDZ domain of Lim Kinase, thus promoting the phosphorylation of cofilin bound to the other, concave side of *Wdr1*.

Secondly, and particularly in the presence of the highly abundant coronin 1a, which helps cofilin to bind cooperatively to F-actin, *Wdr1* deficiency is predicted to favor a full coverage of F-actin by cofilin, for in absence of *Wdr1*, cofilin covered actin filaments are stable (Elam et al., 2013; Gressin et al., 2015; Jansen et al., 2015). The sum of these effects would lead, upon *Wdr1* deficiency, to an excess of stable actin filaments, possibly all covered by cofilin and coronin.

We found that the adverse effects of *Wdr1* deficiency on zebrafish neutrophils can be fully rescued by knocking down expression of their main coronin, coronin 1A. This effect may seem paradoxical at first since coronins and *Wdr1* are both thought to help cofilin sever F-

actin. Yet we envision two ways in which Coro1A depletion could counteract the adverse effects of Wdr1 deficiency.

Firstly, while the three factors together are considered to be the optimal combination for F-actin severing, recent data (Jansen et al., 2015) indicate that coronin binds to F-actin first, enhances subsequent cofilin binding, and maximal severing occurs after Wdr1 also binds. In the absence of both Wdr1 and coronin, actin filaments are thus predicted to be less saturated with cofilin, thereby bringing the latter's density closer to optimal for F-actin severing without Aip1/Wdr1 help (Fig. 8). In addition, coronin 1A has been found to stabilize actin filaments by stapling them together (Galkin et al., 2008), and that effect would be suppressed upon Coro1a depletion.

Secondly, coronin 1B has been shown to interact with and activate Slingshot phosphatase, thus promoting cofilin dephosphorylation (Cai et al., 2007). That property may be shared by coronin 1A, for its depletion in COS cells has been observed to increase levels of p-cofilin - and interestingly also of pMLC and cell contractility (Ojeda et al., 2014). Still, given the delicate, fine tuning of the actin dynamics pathways involved in leukocyte cell migration, it is striking to witness that coronin 1A depletion not only rescued neutrophils from death, but that their full amoeboid motility was restored. This result will be an important datapiece to consider for any future modelling of leukocyte amoeboid motility in vivo. Meanwhile, the complete restoration of Wdr1-deficient neutrophils by coronin 1A depletion may open a new prospect for the treatment of LLS patients. Moreover, since the *WDR1* mutations found in LLS patients are presumably hypomorphic relative to the carmin mutation, an even milder manipulation of coronin 1A expression or activity might well suffice to correct the disease.

Materials and methods

Zebrafish Lines and Maintenance

Wild-type AB and transgenic stocks of zebrafish were maintained as described (Westerfield, 2000). Embryos were maintained in 0.003% 1-phenyl-2-thiourea (PTU) to inhibit pigmentation for maximum transparency, and anesthetized with 0.016% (w/v) Tricaine before experimental manipulation. The following transgenic lines were used: Tg(mpx:GFP)ⁱ¹¹⁴ (Renshaw et al., 2006), Tg(lyz:EGFP)^{nz117} and Tg(lyz:DsRed2)^{nz50} (Hall et al., 2012), and Tg(lyz:H2b-mCherry)^{ump7} (G. Lutfalla, CNRS/University of Montpellier, unpublished). Their details can be accessed on the ZFIN database (zfin.org) via their abovementioned allele names.

Generation and identification of the *carmin* mutant

The *carmin* mutant was identified in a large-scale *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen (Tübingen 2005 Screen Consortium) wherein Sudan black staining (Le Guyader et al., 2008) was used to screen for neutropenic mutants. Meiotic mapping of *carmin* was performed using standard simple sequence length polymorphisms. The *carmin* gene was PCR amplified from cDNA made from mutant and wild-type siblings (primers: forward ‘TTCAGCTCCGTTTCAGCCGTTATTC-3’ and reverse 5’-ACGCTGAGCTCTGCTTCTGGAATG-3’). The amplicons were then further amplified using nested primers (forward TACCGGAGGAGAACACGCAGACAT, reverse TGAGGAGGAGAGTGGAACATCAGA, and reverse TCTGAGCTGTTGAGGAGGAGAGTG). Amplified DNA was then purified by PEG precipitation and sequenced (primers: TACCGGAGGAGAACACGCAGACAT, TGAGGAGGAGAGTGGAACATCAGA, CAATCTCCCCACTGAAGAA, AGAAGGTGGTCACGGTGTTTC). Genomic exon sequencing was carried out by amplifying *Wdr1* exon 11 (AGCGAGTTCTACGGGCATC, TGGCAGCTTGATCCTCTTCT, AGTGAGTTCTACGGGCATC, CGGCAGCTTGATCCTCTTCT), and cloning the fragments into a Topo TA vector.

Genotyping

Melt curve genotyping was performed as previously described (Palais et al., 2005), wherein PCR primers were designed bracketing the *carmin* mutation: (5’-CGAAGGTGGTGTGTCTGTCC-3’ and reverse 5’-TGGTGGCGAAGTGCTCATTA-3’). Melt curves were generated either with an Idaho technology plate reader or in a PCR machine.

Confocal Fluorescence Imaging

Zebrafish embryos were anesthetized with 0.02% tricaine, and embedded in 1% low melting point agarose/volvic H₂O in glass bottom μ -dishes at RT. Images were captured using Leica SP8 inverted and SPE inverted and upright confocal microscopy set-ups; the objectives used were HC PL APO CS2 20x/0.75 and 40x/1.10 water immersion objectives on the SP8 set-up, PL Fluotar 16x/0.50 and ACS APO 40x/1.15 oil immersion objectives on the SPE inverted set-up, and a HCX APO L U-V-I 20x/0.50 water immersion objective on the SPE upright set-

up. To generate images of whole embryos, a mosaic of (512x512 pixels) confocal z-stacks of images taken with the 20x objective of the SP8 set-up were stitched together using the Mosaic Merge function of the LASx software.

Pharmacological inhibition of Rho-Rock signaling

2 dpf and 2.5 dpf embryos were dechorionated and placed in 100-200 μ M Rockout (Rho kinase inhibitor III) or control DMSO/Volvic water, in 6-well plates and kept at room temperature (RT). At intervals of 8, 12, 16, and 24 hrs post incubation (hpi), they were mounted in 1% low melting point agarose (supplemented with tricaine/H₂O with or without Rockout) and imaged via the Leica SP8 confocal microscopy set-up.

Morpholino and plasmid injections

Morpholino oligos (Gene Tools) were injected into one to two-cell stage embryos (1.2 nl / embryo) at the concentration indicated in the figures. We used two splice-blocking morpholinos against the *wdr1* gene - 'wdr1 E3' (ACTCACACTCACTCACCGCCCGATG) and 'wdr1 E4' (GTCCAGCAGCGGTCACACTTCTC), two morpholinos to target the *coro1a* gene, a translation blocking morpholino 'coro1a ATG' (ACCTTCCTAGACATGATGACCTGAG) and a splice blocking morpholino, 'coro1a xi3' (ATGAAGTCCTTGTCACCTACCATGA), and a standard control morpholino (CCTCTTACCTCAGTTACAATTTATA).

For transient expression in developing embryos, plasmids containing *mpx:Lifeact-Ruby* or *lyz:H2b-mCherry* transcription units and Tol2 transposition sites (Yoo et al., 2010, 2012) were co-injected with Tol2 transposase mRNA into Tg(*mpx:GFP*) embryos at the 1-cell stage (2.5-5 pg/embryo).

Whole-mount immunohistochemistry and TUNEL staining

We used rabbit anti-phospho(ser19)-myosin light chain antibody (1:25, ref. 3671S, Cell Signaling); mouse anti-lamin B2 (1:25, ab8983, Abcam); rabbit and mouse anti-cofilin (1:25, SAB4300577, SAB2702206), rabbit anti-phospho(ser3)-cofilin (1:25, SAB4300115) from Sigma-Aldrich, and phalloidin Alexa Fluor 647 or Alexa Fluor 488 (1:10, A22287 and A12379, Molecular Probes). The secondary antibodies (used at 1:300) were Alexa Fluor 488 conjugated goat anti-mouse (ThermoFisher, ref. A11017), Cy3 conjugated goat anti-mouse and anti-rabbit (Jackson ImmunoResearch, refs. 115-166-003 and 111-166-003). Whole embryos were fixed in 4% formaldehyde/microtubule stabilizing buffer (MSB) for either 2-4 hrs at RT or overnight at 4°C, then quenched with NaBH₄/PBS as described previously (McMenamin et al., 2003). After washes in PBSDT (PBS, 1% DMSO, 0.1% Tween), they were treated with H₂O/0.1% Tween for 30 min at RT, acetone at -20° for 20 min., rinsed with 1xHBSS/0.1% Tween, permeabilized with collagenase solution (Svoboda et al., 2001) for up to 1.5 hr, washed, blocked with 10% Western blocking solution (WBS)/PBSDT, and then incubated 24-48 hrs at 4°C with the primary antibodies. Following washes, embryos were blocked in 10% WBS/PBSDT again before 24-48 hrs incubation in secondary antibodies at 4° with agitation. After multiple washes, Hoechst 33342 (2 μ g/ml) or RedDot 1 (1:200, BTM40060-T, Biotium) was applied for 30-60 min at RT, and the embryos were washed

again in PBSDT. They were then embedded in either 1% low melting point agarose/ H₂O or mixed 1:1 with Mowiol 4-88 (Fluka, #8138) in glass bottom μ -dishes for confocal imaging. The specificity of the anti-cofilin and anti-phospho-cofilin antibodies in the zebrafish embryos was confirmed by the following observations: the p-cofilin and whole-cofilin antibodies both labelled periodic striations in somite muscles intercalated with phalloidin / pMLC labelled striations. In addition, the whole-cofilin - but not the p-cofilin – antibodies delineated periderm cell contours (as does phalloidin), and quite strongly the characteristic F-actin microridges at the surface of these cells, indicating that in these structures, cofilin is in non-phosphorylated (hence potentially actin-binding) form - consistent with the fact that these actin microridges were shown to be dynamic (Lam et al., PLoS One 2015). TUNEL staining was performed using the Apoptag Red In situ Apoptosis Detection Kit (Merck Millipore).

Image analysis and statistics

For quantifying neutrophil numbers in live embryos as in Fig. 1B, lyz:GFP+ neutrophils were manually counted on maximum projection images from mosaic confocal z-stacks of whole embryos obtained by stitching tile scans obtained with the 20x objective.

For the quantitative analysis of immunohistochemically stained embryos, confocal image z-stacks acquired and then deconvoluted via the Lightning deconvolution system implemented in the LASx software driving the Leica SP8 set-up were then analysed with the Imaris software (Bitplane). Individual neutrophils were segmented in 3D from their histological environment based on their GFP content, and their content in phalloidin/F-actin, cofilin, p-cofilin, and pMLC was quantified via the 3DView module. The colocalisation of F-actin with the immunolabeled molecule was then analysed within each segmented neutrophil via the Colocalisation module. The Pearson correlation coefficient was calculated first; then if it was > 0 , the Costes thresholds and then the Manders A and Manders B coefficients were calculated (Manders A = fraction of F-actin colocalising with immunolabeled molecule intensity above the Costes threshold calculated for the latter, and vice versa for Manders B). Graphpad Prism was used for statistical analysis of all the data, using the tests indicated in the Figure Legends.

Online supplemental material

Fig. S1 (related to Fig. 1) shows that a retroviral insertion *wdr1* mutant does not complement the *carmin* mutant for the loss of neutrophils, confirming that the *wdr1* mutation in *carmin* is the causative mutation. Fig. S2 (related to Fig. 2) shows the overall phenotype of the mutant beyond the loss of neutrophils. Fig. S3 (related to Fig. 3) shows that like their WT siblings, neutrophils of *carmin* mutants have one and only one centrosome. Fig. S4 (related to Fig. 7) shows that both a splice blocking and a translation blocking morpholinos rescue neutrophils in *Wdr1*-deficient embryos. Video 1 (related to fig. 1) shows by time-lapse confocal imaging the gradual decrease and reduced mobility of neutrophils in a whole *carmin* embryo compared to a WT sibling. Video 2 (related to Fig. 2) shows by time-lapse confocal imaging of embryos expressing Lifeact-Ruby in neutrophils the intense F-actin clumps and reduced mobility of neutrophils in a *carmin* embryo compared to a WT sibling. Video 3 (related to Fig. 3) is confocal z-stack through a mutant neutrophil illustrating that their nucleus becomes

multiglobular. Video 4 (related to Fig. 3) shows by in vivo time-lapse confocal imaging in mutant and WT sibling embryos of neutrophils expressing H2b-mCherry the unusual nuclear/chromatin fluidity or unwinding in mutant neutrophils, followed by their eruption. Video 5 then shows in more detail the successive stages of chromatin lengthly unwinding, then rapid condensation, followed by cell fragmentation; Video 6 then shows that macrophages in the vicinity of neutrophils engulf neutrophils only once they have reached the stage of chromatin condensation. Video 7 (related to Fig. 4) shows through a confocal z-stack three mutant neutrophils with still uncondensed nucleus/chromatin that display no lamin B2 stained NE. Video 8 (related to Fig. 5) shows via time-lapse confocal imaging of a Rockout-treated WT embryo that neutrophils undergo a pathology similar to that seen in *carmin* embryos. Video 9 (related to Fig. 7) shows by time-lapse imaging that coronin1a knock-down fully rescues neutrophils in *carmin* embryos.

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Author contributions: M. Redd and P. Herbomel conceived the project. E. Murayama isolated the *carmin* mutant and M. Tauzin conceived and performed its initial phenotypic characterization. M. Redd, M. Yousfi, and C. Bowes conceived, performed and analysed the results of most experiments. C. Bowes and P. Herbomel wrote the manuscript. P. Herbomel analysed the data, supervised the project and obtained its funding.

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Figure Legends

Figure 1. Wdr1 is necessary for the maintenance of neutrophils in developing zebrafish.

(A) Sudan black-stained 3 dpf *carmin* embryos and siblings reveal a large reduction in neutrophils in *carmin* mutants. Scale bar, 100 μm . (B) 50 live *Tg(lyz:GFP) carmin* and sibling embryos were imaged by confocal fluorescence microscopy (left), and the total number of neutrophils was counted in a subset at several stages between 1.5 dpf/36 hpf and 3 dpf/72 hpf (right). Statistical significance of the difference between mutant and sibling embryos was determined by one-way ANOVA, with Sidak's multiple comparisons test; (ns), $p > 0.05$; (**), $p \leq 0.01$; (***), $p \leq 0.001$. Data shown as mean \pm SD. Scale bar, 200 μm .

(C) Mutation mapping (left) and subsequent sequence analysis (right) revealed a W to R substitution at position 540, close to the C terminus of Wdr1. (D) Number of neutrophils at 56 hpf in the caudal half (from mid-trunk to tail tip) of WT embryos injected with either of two splice-blocking morpholinos against Wdr1 ($n=16$ for each category), all from the same experiment (no pooling); this experiment was repeated at least four times.

Figure 2. Wdr1 deficiency leads to the accumulation of F-actin in the *carmin* mutant.

(A) F-actin (green) and neutrophils (red) in the tail of *Wdr1^{carmin}* vs. *Wdr+* sibling embryos (dorsal up, rostral to the right); arrows point at cortical/sub-cortical F-actin aggregates in epidermal cells of the caudal fin in mutant embryos. (B) F-actin in neutrophils. In WT neutrophils F-actin is observed diffuse in the cytoplasm and delineating the cell cortex, whereas in the mutant it accumulates as intracytoplasmic clumps. (C) Timelapse confocal imaging of *Tg(mpx:GFP) carmin* and WT sibling embryos injected with a *mpx:Lifect-ruby* plasmid also reveal intense and only slowly remodeling F-actin clumps in the mutant neutrophils; time is indicated in hrs:min:sec (see also Video 2). Scale bars, 100 μm (A) and 10 μm (B, C).

Figure 3. Unusual characteristics of the Wdr1-deficient neutrophil nucleus.

(A) Hoechst staining of *Tg(lyz:DsRed)* embryos reveals that unlike WT, mutant neutrophils display a multiglobular nucleus. (B) Timelapse imaging of *Lyz:H2b-mCherry* in *Tg(mpx:GFP)* mutant embryos documents a striking, continuous unraveling of the chromatin of neutrophils over the course of 9 hrs, after which chromatin rapidly condenses in several clumps and the cell erupts; see also Videos 4, 5 and 6. (C) TUNEL staining of mutant vs. WT GFP+ neutrophils by 54 hpf. The second row shows a mutant neutrophil with an already deformed nucleus, but no TUNEL staining, nor GFP depletion from the nucleus ($n=6$). 25% of mutant neutrophils are TUNEL+ by then; the third and fourth rows show an example (two confocal planes of the same neutrophil); in this case the nucleus appears multiglobular, each globule is TUNEL+ and also displays enhanced Hoechst staining due to DNA condensation, and GFP depletion relative to the cytoplasm (arrow) ($n=24$). Scale bars, 10 μm .

Figure 4. Wdr1-deficient neutrophils lose their nuclear lamina.

Whole-mount immunofluorescence images of neutrophils in sibling vs mutant *Tg(Lyz:GFP)* embryos at 2.5 dpf immunostained for Lamin B2 (red), with DNA stained by the far-red fluorescent dye RedDot 1 (cyan). Whereas lamin B2 delineated the nucleus in neutrophils of

WT siblings (**A**, arrows), it did not in any L-stage mutant neutrophil (**C,D**), nor in some at E-stage (**B**; see also Video 7). Aggregates of lamin B2 were often apparent instead (**D**, arrow). (**E**) Proportions of mutant (n=13) vs. WT (n=16) neutrophils in which a full lamin B2 delineated NE was detected. Chi square test; (*) $p < 0.05$. Scale bar, 5 μm .

Figure 5. Acto-myosin perturbation plays a key role in the nuclear instability of Wdr1 deficient neutrophils.

(**A,B**) Whole-mount immunofluorescence confocal images of transgenic 2.5 dpf Tg(lyz:GFP) *carmin* mutant and WT sibling probed with s19-pMLC antibody (red) and phalloidin (cyan). (**A**) In neutrophils of sibling embryos, pMLC appears both cortical -together with phalloidin-labelled F-actin- and diffusely cytoplasmic. (**B**) In mutants, intense ‘caps’ of pMLC appear at the periphery of neutrophils, quite apart from their phalloidin-labelled cytoplasmic F-actin clumps.

(**C, D**) Quantification of the mean intensity (**C**) and colocalisation (**D**) of pMLC and F-actin in the 3D extent of individual mutant (n=12) vs. WT sibling (n=12) neutrophils, all from the same experiment. For the Manders' coefficients, A is F-actin, B is pMLC. For statistical analysis, Welch's t test was used; (****) $p < 0.0001$.

(**E-J**) WT Tg(mpX:GFP; lyz:H2b-mCherry) embryos were incubated in 200 μM Rockout at 2 dpf for 8-16 hours. (**E**) Immunostaining confirmed decreased amounts of p-MLC upon Rockout treatment. (**F,G**) Live confocal imaging revealed within the neutrophils of Rockout treated embryos a reproduction of the characteristic chromatin (red) unwinding (**F**, arrow) and cell fragmentation (**G**) seen in neutrophils of Wdr1-deficient embryos; (**G**) shows selected time points of the time-lapse sequence shown in Video 8; time is indicated in hrs:min. (**H, I, J**) Immunostaining for Lamin B2 (red) in control (**H**, n=24) vs. Rockout treated embryos (**I**, n=25) evidenced loss of NE in some neutrophils after Rockout treatment (**I,J**); all points are from the same experiment; for statistical analysis, a Chi square test was used; (**) $p < 0.01$. Scale bars, 5 μm .

Figure 6. Cofilin is constitutively active in Wdr1-deficient neutrophils

Whole-mount immunodetection in 2.5 dpf Tg(lyz:GFP) *carmin* and sibling embryos of whole cofilin (**A-D**) and Ser3-phospho-cofilin (**E-H**). (**A**) In neutrophils of WT sibling embryos, cofilin is detected throughout the cytoplasm with some enrichment at the cell cortex (arrow) clearly delineated by F-actin (cyan). (**B**) In mutant embryos, cofilin forms aggregates that correlate with the F-actin cytoplasmic clumps (arrows). (**C, D**) Quantitative analysis of the mean density (**C**) and colocalisation (**D**) of pMLC and F-actin in the 3D extent of individual mutant (n=25) vs. WT sibling (n=15) neutrophils, all from the same experiment. For the Manders' coefficients, A is F-actin, B is cofilin. For statistical analysis, Welch's t test was used. (**E**) In the neutrophils of sibling embryos, phospho-cofilin was readily detected as a diffuse signal throughout the cell, whereas in the neutrophils of mutant embryos (**F**), p-cofilin was not visible. (**G, H**) Quantitative analysis of the mean density (**C**) and colocalisation (**D**) of p-cofilin and F-actin in the 3D extent of individual mutant (n=18) vs. WT sibling (n=19) neutrophils, all from the same experiment. For the Manders' coefficients, A is F-actin, B is p-cofilin. For statistical analysis, Welch's t test was used. Scale bars, 5 μm .

Figure 7. Coronin1a depletion fully rescues Wdr1-deficient neutrophils.

(A) Images of 2.5 dpf whole-mount DAPI-stained Tg(lyz:GFP) Wdr1+, Wdr1-/-, and Wdr1-/- embryos injected with a coronin1a translation-blocking morpholino. Coronin1a knockdown has restored the nuclear integrity of mutant neutrophils compared to the fractionated nuclei of untreated embryos (arrows). (B) Phalloidin staining of neutrophils in *coro1a* Mo injected Wdr1-/- embryos shows that their typical shape and cortical location of F-actin has been restored; the nucleus also remains GFP-enriched. (C,D) Control or morpholino-injected *carmin* and sibling embryos were mounted in agarose by 26 hpf, time-lapse imaged in parallel for 20-24 hrs via a wide-field microscope, and later genotyped; (C) Representative images of sibling, mutant, and *coro1a*-ATG Mo injected mutant embryos after 15 hrs of time-lapse imaging (see also Video 8). (D) Neutrophil counts over time in embryos of the three genotypes, either uninjected (upper graph, n=39), or injected with the *coro1a*-ATG Mo (lower graph, n=40), as derived from the time-lapse movies. All points are from the same experiment. (E) Neutrophil counts at 56 hpf in the caudal half of embryos injected with control (n=42) or *coro1a*-ATG morpholino (n=40), and later genotyped. All points are from the same experiment. Statistical significance was determined by one-way ANOVA, with Tukey's multiple comparisons test, $p < 0.05$; (***) $p \leq 0.001$. Differences between injected WT and rescued mutant embryos are statistically non significant. Scale bars, 5 μm (A), 10 μm (B), and 100 μm (C).

Figure 8. A model of F-actin severing in Wdr1-deficient vs. Wdr1-proficient neutrophils and rescue of the former by coronin1a depletion.

(A) Coronin 1 binding increases recruitment of cofilin, which in turn recruits Wdr1 (Jansen et al., 2015). When cofilin is present in intermediate concentrations, it moderately severs actin filaments, at transition sites between cofilin-bound and -bare F-actin, without potentiation by Wdr1. (B) In high cofilin concentration conditions, cofilin instead stabilizes actin filaments. However, Wdr1 binding to the cofilin-bound actin filaments triggers fast severing of the filaments (Ono, 2017). (C) In Wdr1-deficient neutrophils, coronin 1A binds actin filaments, and recruits cofilin. In absence of Wdr1, cofilin is fully unphosphorylated and thus fully coats and stabilizes F-actin. (D) When Wdr1-deficient embryos are depleted of coronin 1A, the absence of coronin 1A on F-actin lowers the binding of cofilin. Moderate concentrations of bound cofilin, despite the absence of Wdr1, can now sever actin at moderate rates.