F-actin flashes on phagosomes mechanically deform contents for efficient digestion in macrophages

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DOI: 10.1242/jcs.239384

Editor: Daniel Billadeau

Review timeline
Original submission: 18 September 2019
Editorial decision: 14 October 2019
First revision received: 8 April 2020
Accepted: 11 May 2020

Original submission

First decision letter

MS ID#: JOCES/2019/239384

MS TITLE: F-actin flashes on phagosomes mechanically deform contents for efficient digestion in macrophages

AUTHORS: Mathieu B. Poirier and Rene E. Harrison
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. In general, the three reviewers critiques suggest that the manuscript provides important mechanistic insight. However, it is clear that some IF images require further quantification (R1 and R2) and that the function of Arp2/3 could be further characterized (R1 and R2). There is also a suggestion to use live or heat-killed bacteria in your study of F-actin flashes or pharmacologic manipulation of them, to demonstrate the physiological significance of the process. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The manuscript by Poirier and Harrison studies the function of actin recruitment to the maturing phagosome and elegantly shows that cyclic actin recruitment and depolymerization contributes to the digestion on ingested non-rigid substrates.
Using life-act in live cells, the authors show that this novel mechanism of recurrent actin recruitment and polymerization requires activation of RhoA, is ROCK kinase-dependent but PIP2 independent. They also demonstrate that the mechanism is required to induce particle deformation, delays maturation and accelerates proteolysis, a mechanism that requires Myo IIa. Overall, this is an elegant paper that increases our mechanistic understanding of one of the most important innate immune mechanisms, i.e, phagocytosis.

Comments for the author

Specific comments
1) Actin flashes requires RhoA and Rock. Although the authors speculate on how this mechanism may be possibly regulated, in similar fashion as RhoA GAP/GEF temporal activities during actomyosin assembly in epithelial cells, it would be interesting if they could identify or speculate which RhoA-GAP may associate with phagosomes to mediate RhoA activation cycles. Alternatively since RhoA seems to be enriched in phalloidin-enriched phagosomes, they should clarify whether RhoA is inactivated at, or just removed from the non-flashing phagosomes. The use of RhoA-biosensors at flashing and non-flashing phagosomes would be helpful to clarify some of these points.

2) While they show that Arp2/3 is recruited to the flashing phagosome, the authors felt short in demonstrating whether this process is essential for actin flashing, deformation or proteolysis. This could be studied with the use of Arp2/3 inhibitors.

3) While the authors demonstrate that talin recruitment is synchronized with actin flashes and discuss the critical link between integrins and actin filaments, their results do not explain why or how integrin signaling is intermittently coordinated all around the phagosome to induce these actin polymerization cycles. Does substrate deformation induce engagement and dis-engagement of ligand and receptors? Or is the signaling interrupted and reactivated downstream of ligand-receptor interaction? Can the activation states of the integrin at flashing phagosomes be established using antibodies that specifically detect this activation state?

4) Because of the significance given to talin recruitment in the discussion Talin cyclic recruitment (and RhoA recruitment) should be studied in further detail and quantified.

5) Figure 2 legend should be revised as it refers to Figure 7 when describing ROCK inhibition.

Reviewer 2

Advance summary and potential significance to field

The manuscript by Poirier and Harrison analyzes periodic flashes of F-actin association with fully formed phagosomes inside macrophages. The work confirms and extends earlier studies, first by Yam and Therioux (2004. MBC)) and later by Liebl and Griffiths (2009. JCS), which described actin flashing on phagosomes containing solid particles and on macropinosomes. Of note, the present study analyzes flashes on phagosomes containing deformable sheep red blood cells and shows that flashes correlate with deformations of the phagosome mediated by actomyosin contraction. Additionally, phagosomes with flashes exhibit measurable enhancement of lysosomal degradation, which leads to the suggestion that actin-based deformations provide a kind of ‘mastication’ that enhances particle degradation.

Comments for the author

The data support this interesting conclusion.

Major concerns:
1. Immunofluorescence is used to localize proteins associated with integrin-based motility. Images are presented to support the positive associations but no quantitative measurements are provided to support these examples. How many images were analyzed? What was the frequency of antigen association with actin-positive phagosomes vs. actin-negative phagosomes?

2. The actin-positive phagosomes contained myosin IIa and phagosome distortions were significantly inhibited by the myosin II inhibitor blebbistatin, suggesting a role for myosin II in the process. Actin-positive phagosomes also contained Arp2/3 components, which are known to contribute to comet-tail formation and organelle propulsion like that seen with flashing phagosomes. Therefore, this study should examine more thoroughly the contribution of actin polymerization to the phagosome deformations. To what extent to Arp2/3 or formins contribute to the deformations?

3. The analysis of the role of flashes in phagosome-lysosome fusion was thoroughly analyzed by Liebl and Griffiths. The present study examines the same process less thoroughly and comes to similar conclusions, which makes those data somewhat superfluous.

Minor concern:

4. The supplementary video provides a good illustration of the F-actin flashes. However, it should include a time-stamp and scale bar.

Reviewer 3

Advance summary and potential significance to field

This manuscript describes a novel, post-internalization role for F-actin on phagosomes during complement receptor (CR)-mediated uptake of opsonized particles. While F-actin is needed for initial, external phagocytic cup formation, it is normally, rapidly depolymerized upon internalization and sealing of the nascent phagosome.
The authors of this study noticed recurrent waves (flashes) of F-actin appearing specifically on CR-mediated phagosomes inside cells after uptake. The F-actin flashes correlated with the phagosomal staining of focal adhesion and actin polymerizing machinery including talin, FAK, Wasp, myosin II, consistent with integrin- and F-actin associated processes. The flashes also correlated with deformation of soft but not rigid particles, the delayed recruitment of endosomes and increased degradation as measured with a proteolytic fluorescent marker. Overall the study makes a nice case in characterizing the F-actin flashes and proposing them as an additional feature of phagocytosis that could, for instance, help in the destruction of complement-opsonized pathogens.

Comments for the author

This group is well-versed in the study of phagocytosis and accordingly these experiments are executed and analyzed in a rigorous and convincing fashion.

Having described these new phagocytic flashes, it would add significantly to the impact of the study to include some additional data to demonstrate a relevant context or function for the flashes. Extensive additional experiments would not be needed, but perhaps new data could specifically show that flashes do occur on CR-phagosomes after uptake of live or killed bacteria (to confirm that this is an anti-microbial process), or the authors could show that blocking F-actin flashes (e.g. perhaps by cytochalasin after internalization to block the flashes or blebbistatin to block the ‘chewing’) has longer term consequences in the cells, for instance by blocking clearance of bacteria or SRBC.

Comments.
In Figure 6 the panel numbering is misaligned in the text and the authors may have intended to add another data panel here?
Also the correlative EM in Figure 6 is very striking and it would be informative to show a slightly wider view of the TEM panel to fully include the non-flash phagosomes with high magn insets to show their phagocytic membranes.

First revision

Author response to reviewers' comments

Dear Dr. Billadeau,

Thank you for the referee's comments and your encouragement to submit a revised manuscript to the *Journal of Cell Science*. We found the comments raised by the Reviewers to be extremely relevant and constructive. Each individual comment has been addressed below. We hope that you find that the modifications we have made to the manuscript, and the additional data we have included, sufficiently address these comments to consider the manuscript for publication.

Response to referee comments (Reviewer comments in italics)

Reviewer 1 Comments

1) Actin flashes requires RhoA and Rock. Although the authors speculate on how this mechanism may be possibly regulated, in similar fashion as RhoA GAP/GEF temporal activities during actomyosin assembly in epithelial cells, it would be interesting if they could identify or speculate which RhoA-GAP may associate with phagosomes to mediate RhoA activation cycles. Alternatively, since RhoA seems to be enriched in phalloidin-enriched phagosomes, they should clarify whether RhoA is inactivated at, or just removed from the non-flashing phagosomes. The use of RhoA-biosensors at flashing and non-flashing phagosomes would be helpful to clarify some of these points.

We appreciated the ideas and opportunity to further understand RhoA signaling on F-actin flashing phagosomes. As recommended, we obtained RhoA biosensors to understand its activity on phagosomes. We were unsuccessful using the EGFP-Anillin construct (Piekny and Glotzer, 2008), which transfected poorly and did not localize to sites of active actin polymerization. We had better luck expressing the GFP-rhotekin GBD (rGBD) construct (Benink and Bement, 2005) which we co-transfected into RAW 264.7 cells stably expressing LifeAct-RFP. With live cell confocal imaging, we consistently observed near-simultaneous recruitment of the RhoA biosensor at the time of F-actin accumulation on the phagosomes. We have now included a supplementary movie (Movie 3) and image stills (Fig. 4C) of a representative movie. To check if expression of the biosensor was influencing F-actin flashing on phagosomes we also did some analysis of the flashing kinetics in cells co-transfected with GFP-rGBD and LifeAct-RFP (Fig. 4D and 4E). The time of F-actin accumulation on the phagosome was similar to the flashes observed on CR-phagosomes in cells only expressing LifeAct-RFP (Fig. 4D). There was a slight, but not significant, increase in the time between flashes in cells co-expressing GFP-rGBD, compared to cells expressing LifeAct-RFP alone (Fig. 4E). This data largely corresponds with our immunofluorescence data of RhoA, which we have now quantified (Fig. 4B) where we observe almost exclusive recruitment of RhoA on phalloidin-positive phagosomes, versus non-flashing phagosomes where RhoA recruitment was not observed. Quantification of immunofluorescence data was also recommended by Reviewer 2.

Our imaging and immunofluorescence data suggests that RhoA is removed from phagosomes when they are not "flashing". To address the mechanism behind RhoA removal from phagosomes, we followed the excellent suggestion to examine the presence of RhoA GAPs on phagosomes. We speculated that p190RhoGAP was a likely candidate, based on its known signaling activities and effectors (Amin et al., 2016; Ridley et al., 1993). We obtained antibodies to p190RhoGAP and

performed immunofluorescence analysis and quantified its presence on F-actin-positive and -negative phagosomes. Immunofluorescence images are shown in Fig. 4A and quantified in Fig. 4B. We discovered that p190RhoGAP prominently labelled phagosomes enriched with F-actin, but not phagosomes without F-actin. This data shows that p190RhoGAP is recruited to phagosomes and may be involved in the down-regulation of RhoA activity and RhoA release from the phagosomes. This is now included in the Discussion on Page 14.

2) While they show that Arp2/3 is recruited to the flashing phagosome, the authors felt short in demonstrating whether this process is essential for actin flashing, deformation or proteolysis. This could be studied with the use of Arp2/3 inhibitors.

Reviewer 2 also recommended that very similar experiments be performed to assess Arp2/3 involvement in F-actin flashing and particle deformation. We used the Arp2/3 inhibitor CK-666 for these detailed analyses. Cells were treated with 150 ¤M of CK-666 15 minutes after C3bi-sRBC internalization to avoid issues with particle uptake in the presence of the drug. For fixed assays, phagocytosis was allowed to proceed for an additional 30 minutes prior to fixation. For live cells assays, the LifeAct-RFP-transfected cells were imaged immediately. We chose these treatment strategies and drug concentration based on 2 papers that used similar cells and timelines (Barros-Becker et al., 2017; Rotty et al., 2017).

We first assessed the prevalence of F-actin flashes on phagosomes in cells treated with CK-666. Shown in Fig. 4H, the percentage of LiveAct-RFP-positive phagosomes in CK-666-treated cells was significantly lower than DMSO-treated cells. This indicates that Arp2/3 is required for detectable F-actin accumulation on phagosomes. As a smaller percentage of phagosomes still recruited F-actin, we monitored these phagosomes for flashing dynamics and particle deformation using live cell confocal microscopy of LifeAct-RFP-transfected cells. A representative movie (Movie 4) as well as still images from the movie (Fig. 4G) are now included in the revised manuscript. As shown in the movie, we saw a very striking and interesting phenotype. In many of the CK-666-treated cells, F-actin was present on phagosomes for an extended period. Approximately 30% of cells showed a single protracted flash during imaging while another third of treated cells showed long flashes as well as extended periods between flashes. When all of the data was accumulated (30 flashing phagosomes from 10 independent movies) and analyzed, the average duration of an F-actin flash on a phagosome was over 10 minutes and the average time between flashes was over 6 minutes in CK-666-treated cells. These values are over double what we have observed in untreated cells (Fig. 2G). We have now shown these CK-666 quantifications in Fig. 4I and 4J of the updated manuscript. While we do not have a molecular explanation for these interesting phenotypes, our interpretation of these results is now included in the Discussion on Page 15. As suggested by the Reviewer, we also quantified sRBC deformation in flashing phagosomes in CK-666-treated macrophages. Only 9/30 F-actin-positive phagosomes showed detectable deformation, as measured using DIC imaging, which is much lower than what we have observed in untreated cells (Fig. 1H). This important new data regarding Arp2/3 involvement in particle deformation is now shown in Fig. 4K of the revised manuscript.

3) While the authors demonstrate that talin recruitment is synchronized with actin flashes and discuss the critical link between integrins and actin filaments, their results do not explain why or how integrin signaling is intermittently coordinated all around the phagosome to induce these actin polymerization cycles. Does substrate deformation induce engagement and dis-engagement of ligand and receptors? Or is the signaling interrupted and reactivated downstream of ligand-receptor interaction? Can the activation states of the integrin at flashing phagosomes be established using antibodies that specifically detect this activation state? Because of the significance given to talin recruitment in the discussion, talin cyclic recruitment (and RhoA recruitment) should be studied in further detail and quantified.

These are terrific questions which we have spent some time pondering. As recommended, we quantified talin recruitment to flashing and non-flashing phagosomes using immunofluorescence, as a proxy for integrin activation. Robust talin recruitment was almost exclusively observed on F-actin positive phagosomes, but absent on F-actin-negative phagosomes (Figs. 3A,B). We wanted to confirm these results in live cells. We initially thought that live cell imaging of talin-GFP would be fairly straightforward but ran into numerous obstacles including the demise of our definite focus laser on our epifluorescent microscope that we use for live cell imaging. Consequently, most of the

revision experiments were performed on a facility spinning disk confocal which has an incubation platform that was adequate for most of the analyses, although the DIC optics were poor.
We also had difficulties with expression of talin-GFP constructs. The first construct we obtained from Addgene (GFP-talin1, #26724) rarely localized to F-actin flashes (see *Fig. 1* below) in transfected cells. We then realized that this construct had GFP fused to the N-terminus of talin, where the majority of the integrin binding domains are located (Klapholz and Brown, 2017), which likely prevents recruitment and binding to integrins.

"NOTE: We have removed unpublished data that had been provided for the referees in confidence."

We next tried transducing RAW 264.7 cells with the BacMam CellLight™ Talin-GFP, from Fisher (catalog # C10611), which is a fusion construct of the C terminus of human talin with emGFP. Although this construct had the fluorophore in a more appropriate location (C-terminus), we found that all of the concentrations that we tried (10 - 50 viral particles per cell) were toxic to RAW 264.7 cells. After a short transduction time for protein expression (approximately 15 hours), we observed reduced cell density and cell rounding and poor localization of the construct. Example images are provided below in *Fig. 2*.

"NOTE: We have removed unpublished data that had been provided for the referees in confidence."

We next looked for a mouse talin1 construct in a mammalian expression vector and obtained mEmerald-talin-N-10 from Addgene (#54266). We had good success with expression of this construct and were able to obtain sufficient movies of F-actin flashing in co-transfected cells before COVID19 shut down the laboratory. The results of these experiments are now shown in Fig. 3C-E and Movie 2. The co-expression of the construct did not markedly influence F-actin flashes, although interestingly, the time between flashes was increased (Fig. 3D-E). The recruitment of mEmerald-talin to phagosomes was near-coincident with the F-actin flashes within the time resolution of our imaging (Fig. 3C, Movie 2). This combined with our new quantification results of talin immunostaining on flashing and non-flashing phagosomes (Fig. 3B), strongly suggest that talin is present at the time of F-actin recruitment and then removed and recruited for the subsequent flash.

Importantly, our new talin data suggests that integrins are likely active in flashing phagosomes. We are currently unable to directly determine the affinity status of CR3 since the only commercially available antibody (CBMR1/5) is against human CR3 and the antigen is within the ligand-binding site so will not recognize particle-adhered CR3 (we discovered this in (Patel and Harrison, 2008)). We did however look at total CR3 on phagosomes using the Mac1 M1/70 monoclonal antibody. The presence of CR3 on phagosomes is almost certainly a main driver of F-actin flashes due to its enrichment on phalloidin-positive phagosomes, now shown and quantified in Fig. 3A and 3B. A small percentage of phalloidin-negative phagosomes were also enriched in CR3 (Fig. 3B) which likely represents phagosomes "in-between" flashing, since it would be very energetically inefficient to remove and redeliver membrane proteins to the phagosome in these rapid time frames.

Together these data suggest that integrin activity / talin recruitment are likely the most upstream elements regulating the F-actin recruitment cycles on phagosomes. How particle deformation relates to integrin activity is still unclear but to gain some insight into this we can examine our sRBC versus latex bead data (Figs. 2G,H). The F-actin flashing disparities we observe between sRBCs and beads can be equated to stiffness differences, if we assume that all other things are equal. The major significant differences that we observed in F-actin flashing kinetics were that deformable targets had more total flashes that reappeared faster on phagosomes (less time between flashes) (Figs. 2G,H). Thus, deformation of the particle seemed to accelerate the "on rate" of flashes over a longer cycling period. This may be due to release and reattachment of integrins to new ligands on the particle as it is manipulated in space, which would otherwise remain fairly static on a rigid particle. As the Reviewer suggested, the engagement and reengagement of integrins could be the major instigator of flashing dynamics however we do not have data directly supporting this.

We have now discussed our data interpretation and the implications of our new results in the Discussion on Pages 13 and 15.

5) Figure 2 legend should be revised as it refers to Figure 7 when describing ROCK inhibition.

This has now been corrected in the manuscript.

Reviewer 2 Comments

1) Immunofluorescence is used to localize proteins associated with integrin-based motility. Images are presented to support the positive associations, but no quantitative measurements are provided to support these examples. How many images were analyzed? What was the frequency of antigen association with actin-positive phagosomes vs. actin-negative phagosomes?

We agree that this important immunofluorescence data is improved with quantification. We have now quantified both F-actin-positive and F-actin-negative phagosomes for the presence or absence of all of the proteins of interest. In this revised manuscript we also include the data for CR3 (at 20 minutes and 40 minutes post-internalization) and p190RhoGAP to address concerns raised from Reviewer 1. As now seen in Figs. 3A and 4A, the presence of these proteins almost always corresponded with the detection of F-actin on the phagosomes.

2) The actin-positive phagosomes contained myosin IIa and phagosome distortions were significantly inhibited by the myosin II inhibitor blebbistatin, suggesting a role for myosin II in the process. Actin-positive phagosomes also contained Arp2/3 components, which are known to contribute to comet-tail formation and organelle propulsion like that seen with flashing phagosomes. Therefore, this study should examine more thoroughly the contribution of actin polymerization to the phagosome deformations. To what extent to Arp2/3 or formins contribute to the deformations?

Establishing a definitive role for Arp2/3 in particle deformation was also raised by Reviewer 1 (see Comment #2). During the revision period, our epifluorescence live-imaging microscope stopped working so we performed most of the revisions on a spinning disk microscope that is equipped with an incubation stage that is adequate for long-term live cell imaging. Reviewer 1 also suggested that we examine F-actin flashing dynamics on phagosomes when Arp2/3 was inhibited with CK-666, as well as the prevalence of F-actin-positive phagosomes. As detailed in Comment #2 to Reviewer 1, both F-actin flashing dynamics as well as sRBC deformation were affected in CK-666-treated cells. In CK-666-treated cells, only 9/30 phagosomes imaged showed detectable deformation, measured using DIC imaging, which is much lower than what we have observed in untreated cells (Fig. 1H). This important new data is now shown in Fig. 4K of the revised manuscript.

3) The analysis of the role of flashes in phagosome-lysosome fusion was thoroughly analyzed by Liebl and Griffiths. The present study examines the same process less thoroughly and comes to similar conclusions, which makes those data somewhat superfluous. The supplementary video provides a good illustration of the F-actin flashes. However, it should include a time-stamp and scale bar.

Yes we agree that these findings overlap conceptually but wanted to show that a similar phenomenon occurs with C3bi-opsonized targets. We have now included time and scale bars on all of our movies in this revised submission.

Reviewer 3 Comments

1) This group is well-versed in the study of phagocytosis and accordingly these experiments are executed and analyzed in a rigorous and convincing fashion. Having described these new phagocytic flashes, it would add significantly to the impact of the study to include some additional data to demonstrate a relevant context or function for the flashes. Extensive additional experiments would not be needed, but perhaps new data could specifically show that flashes do occur on CR-phagosomes after uptake of live or killed bacteria (to confirm that this is an anti-microbial process), or the authors could show that blocking F-actin flashes (e.g. perhaps by cytochalasin after internalization to block the flashes or blebbistatin to block the 'chewing') has longer term consequences in the cells, for instance by blocking clearance of bacteria or SRBC.

We appreciate these suggestions to improve the relevance of our findings. We attempted to block F-actin flashing to examine long-term consequences on macrophage function however cell lifting following cytochalasin D treatment precluded any analysis. We next examined bacteria as suggested by the Reviewer. The original, seminal paper by Yam & Theriot (Yam and Theriot, 2004) examined F-actin flashing kinetics in epithelial cells after invasion of several bacteria including *Listeria monocytogenes* and *Escherichia coli*. As recommended, we wanted to see if complement-opsonization impacted this event within macrophages. We initially tried live cell imaging studies, but the bacteria were too difficult to follow using DIC imaging on the spinning disk confocal. Next, we did extended phagocytosis assays (20 and 40 minutes post-internalization) of C3bi-opsonized *E. coli* and did indeed observe robust recruitment of F-actin to phagosomes containing *E. coli* in macrophages. We have now included this important finding in Fig. 1F of the revised manuscript.

2) In Figure 6 the panel numbering is misaligned in the text and the authors may have intended to add another data panel here? Also the correlative EM in Figure 6 is very striking and it would be informative to show a slightly wider view of the TEM panel to fully include the non-flash phagosomes with high magn insets to show their phagocytic membranes.

These issues have now been corrected in the updated manuscript. The resolution of our TEM camera could not allow us to discern all phagosome membranes, but we have now included a lower magnification TEM view of the cell which includes all of the flashing and non-flashing phagosomes in Fig. 7A.

References for Reviewers

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Second decision letter

MS ID#: JOCES/2019/239384

MS TITLE: F-actin flashes on phagosomes mechanically deform contents for efficient digestion in macrophages

AUTHORS: Mathieu B. Poirier, Cara Fiorino, Thiviya K. Rajasekar, and Rene E. Harrison
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

All my concerns and suggestions have been addressed.

Comments for the author

All my concerns and suggestions have been addressed.
New data have been incorporated and quantified further supporting the author's conclusions.

Reviewer 2

Advance summary and potential significance to field

The manuscript by Poirier and Harrison analyzes periodic flashes of F-actin association with fully formed phagosomes inside macrophages. The work confirms and extends earlier studies, first by Yam and Therioux (2004. MBC)) and later by Liebl and Griffiths (2009. JCS), which described actin flashing on phagosomes containing solid particles and on macropinosomes. Of note, the present study analyzes flashes on phagosomes containing deformable sheep red blood cells and shows that flashes correlate with deformations of the phagosome mediated by actomyosin contraction. Additionally, phagosomes with flashes exhibit measurable enhancement of lysosomal degradation, which leads to the suggestion that actin-based deformations provide a kind of ‘mastication’ that enhances particle degradation. The data support this interesting conclusion.

Comments for the author

The revised manuscript adequately addresses the few concerns I noted in my first evaluation.

Reviewer 3

Advance summary and potential significance to field

This manuscript makes an important addition to the field of phagocytosis. describing dynamic cycles of F-actin polymerisation and its associated machinery on these phagosomes. The revised manuscript has added significant new data on CR3, talin, Arp2/3 and p190RhoGAP, which brings together mechanistic insights into the how the F-actin flashing occurs.

Comments for the author

The authors have carried out extensive new studies, their additional data addresses the major concerns and suggestions and adds to the impact by further describing roles for key actin-modifying proteins.
Quantification is present in all of the figures and the resulting story makes an important contribution to the field at the level of fundamental biology and the role of actin in phagocytosis triggered by integrin-mediated uptake of particles.