nature portfolio

Peer Review File

β2 integrins impose a mechanical checkpoint on macrophage phagocytosis

Editorial Note: This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed most of my previous concerns very well. However, I remain unconvinced by the authors' conclusion that β2 integrins directly bind to the polyacrylamide matrix of the DAAM particle. This conclusion is unusual given the typical binding behavior of β2 integrins with specific biological ligands such as ICAM-1 and iC3b. Although the authors' experiments suggest that serum somewhat inhibits Itgb2 dependent cargo mechanosensing, this alone does not definitively demonstrate direct binding between β2 integrins and the polyacrylamide matrix. The inhibition observed could be due to other factors present in the serum affecting the interaction rather than indicating a direct binding mechanism.

Reviewer #2 (Remarks to the Author):

The authors have largely responded to my previous critiques of their initial submission and I find the manuscript much improved. The vinculin and talin data in particular is an excellent addition and strengthens the work greatly. I also think the serum free experiments and Mn2+ experiments help with their interpretation. I have two small remaining recommendations:

1. It still remains unclear to me how beta2 integrins could bind directly to the DAAM particles. There is no clear binding domain on the acrylamide. Also, the strength of integrin bonds comes largely from their catch-bond behavior, but in the absence of a specific ligand it's more likely to behave like a slip-bond if anything. I understand the data points in the direction of binding directly to the acrylamide, but it is such an odd result that I feel like it's worth mentioning explicitly in the text how odd this result is and that it's still possible there are other explanations.

2. In the final cartoon (Figure 8) there is text suggesting either rapid turnover or stable recruitment of vinculin. As the data in the manuscript is all shown from fixed images, the only determination can be about enrichment of vinculin, and not about the dynamics of individual molecules. If anything, vinculin binding stabilizes talin in its extended conformation (PMIDs: 24714394, 34463480). Also, it's entirely possible that the kinetics of individual vinculin molecules are identical and it's just that there is more present in the stalling phagocytosis. Since this is all speculation about what is

happening with vinculin at these sites, I would suggest removing this text in the figure and perhaps changing the number of vinculin on the stalling side to convey that there is more vinculin present.

Reviewer #3 (Remarks to the Author):

The authors have added new experimental data and points for discussion to the manuscript.

I am satisfied by these changes and recommend publiction in Nat Comms.

As the mechanism presented might be incomplete, I support the addition of an "?" or "factor X" in the final model. The authors have already indicated their willingess to do this in their rebuttal letter.

We thank the reviewers for their astute suggestions about how to improve our manuscript. Below is a point-by-point response to their concerns, with reviewer text in blue, responses in black.

Reviewer #1

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We agree with the reviewer and have altered our Discussion (p 17, paragraph 2) to communicate a higher level of uncertainty about precisely how β2 integrins engage DAAM particles. We believe that this more nuanced assessment better reflects our experimental results.

Reviewer #2

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Please see our response to review #1 above.

2. In the final cartoon (Figure 8) there is text suggesting either rapid turnover or stable recruitment of vinculin. As the data in the manuscript is all shown from fixed images, the only determination can be about enrichment of vinculin, and not about the dynamics of individual molecules. If anything, vinculin binding stabilizes talin in its extended conformation (PMIDs: 24714394, 34463480). Also, it's entirely possible that the kinetics of individual vinculin molecules are identical and it's just that there is more present in the stalling phagocytosis. Since this is all speculation about what is happening with vinculin at these sites, I would suggest removing this text in the figure and perhaps changing the number of vinculin on the stalling side to convey that there is more vinculin present.

We agree with the reviewer and have removed any reference to vinculin turnover from the schematic.

Reviewer #3

The authors have added new experimental data and points for discussion to the manuscript. I am satisfied by these changes and recommend publiction in Nat Comms.

As the mechanism presented might be incomplete, I support the addition of an "?" or "factor X" in the final model. The authors have already indicated their willingess to do this in their rebuttal letter.

We agree and have added a question mark to the Figure 8 schematic to indicate our uncertainty about the exact mechanisms of phagocytic stalling.