Correction: Stoichiometry of Nck-dependent actin polymerization in living cells

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Several months ago it came to our attention that one of the expression constructs used in this publication, which we had obtained as a gift from another laboratory, was incorrect. What we had described as a fusion of GFP with full-length N-WASp was in fact a fusion of GFP with the proline-rich SH3-binding region of N-WASp ("CB6 GFP–N-WASp-polyPRO" originally described in Moreau et al. [2000]). This construct was used in experiments described in Fig. 6 C and Fig. S3 of the original publication. We have now repeated the experiments in Fig. 6 C with a new construct that we have confirmed contains full-length N-WASp, and the results of these new experiments are shown in the revised Fig. 6. The conclusions are essentially unchanged, and the new data fully support the original model presented in Fig. 7. We chose not to attempt to reproduce the experiments shown in Fig. S3 with the new construct, but have relabeled the figure to accurately describe the actual construct used in the experiment shown. We have revised the text of the results to accurately describe the experiments and the implications of the results. Minor changes were also made to figure legends, Materials and methods, and Acknowledgments. Although the conclusions of our study are not significantly affected by these changes, we are sincerely sorry for the mistake in the original publication and apologize for any inconvenience or confusion that it might have caused our colleagues.

A summary of the changes is included here, and the amended figures are included below.

1. New Fig. 6 C: New data for experiments done since publication of the original paper. It replaces the original Fig. 6 C, with no change to other panels of Fig. 6.
2. New Fig. S3: Replaces the original Fig. S3. Image data are unchanged; the only difference is that the label of the left column is changed to "GFP–N-WASp-Pro" from "GFP-NWASp" to correctly identify the construct used in the experiment.
3. Results, p. 650, last paragraph in the left column: References to "GFP–N-WASp" changed to "emerald–N-WASp" to reflect actual construct used in recent experiments.
4. Results, p. 650, right column: Paragraph revised to reflect that experiment shown in Fig. S3 was in fact done with GFP–N-WASp-Pro instead of full-length GFP–N-WASp as originally reported.
5. Figure legend, p. 651: Legend of Fig. 6 changed to reflect use of emerald–N-WASp (not GFP–N-WASp) in new experiments.
6. Materials and Methods, p. 656: Details of the GFP–N-WASp-Pro and emerald–N-WASp constructs actually used are provided.
7. Acknowledgments, p. 656: Changed to reflect actual construct received from M. Way.
8. Supplemental figure legend, p. S3: Legend of Fig. S3 changed to reflect that GFP–N-WASp-Pro instead of GFP–N-WASp was used in this experiment.

The HTML and PDF versions of this article have been corrected. The errors remain only in the print version.

We would also like to discuss the results published in the original Fig. 6 C, in light of our current understanding that the GFP-tagged construct used contained only the proline-rich region of N-WASp. The fact that the proline-rich region would bind to Nck SH3 domain aggregates in WIP wild-type (WT) but not WIP knockout (KO) cells is rather puzzling, as in vitro binding studies using purified proteins have shown that this region of N-WASp is sufficient to bind to Nck SH3 domains (Rohatgi et al., 2001). Several factors may be at play here. First, multiple groups have shown that WIP expression is important to stabilize endogenous WASp (Chou et al., 2006; Sawa and Takenawa, 2006; de la Fuente et al., 2007), and thus may also affect N-WASp stability and/or localization. Consistent with this, the WIP KO cells are quite sickly, with a number of actin-related defects (Ramesh et al., 2014). Furthermore, we have noted that high-level expression of N-WASp-Pro was rather toxic in WIP KO cells and as a result the average expression level of GFP–N-WASp-Pro was ∼50% higher in WT cells compared to WIP KO cells, based on fluorescence intensity. Not only did the KO cells lack WIP, but they also typically expressed less GFP–NWASp-Pro than the controls. It is important to note that others have reported that GFP–N-WASp-Pro does not bind detectably to vaccinia virus–induced comets (either in the presence or absence of WIP), even though Nck is present at high levels on those comets (Moreau et al., 2000). This suggests that recruitment of GFP–N-WASp-Pro to Nck in vivo is close to the limit of detection, and thus could easily be affected by factors such as differences in its expression level and the presence/absence of other components in the WIP KO cells versus WT controls. Importantly, we did not see enhanced toxicity upon expression of full-length GFP–N-WASp in WIP KO versus control cells, and we were therefore able to use WT and KO cells with similar GFP–N-WASp expression levels for imaging; so this is not a concern with the revised figure.

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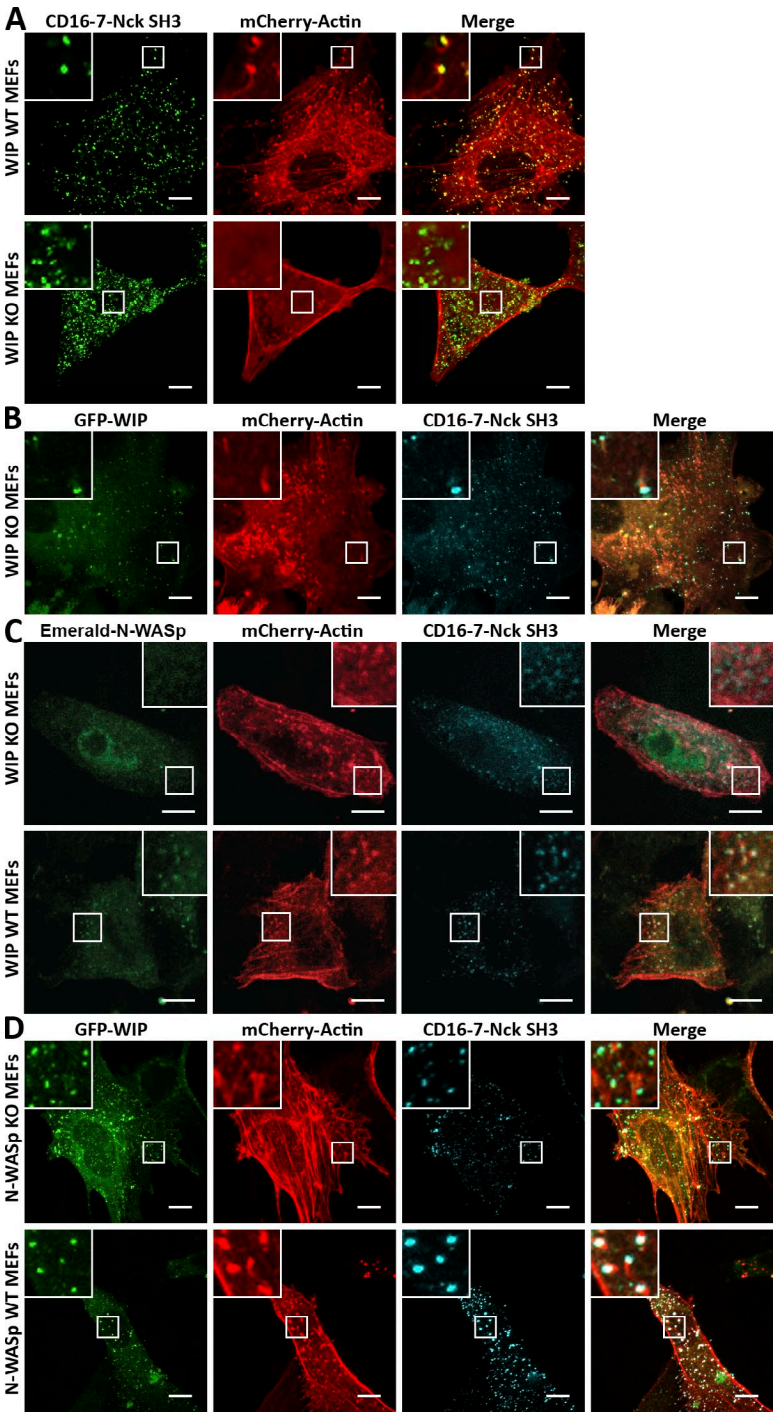

Figure 6. **WIP is an essential component of the Nck–N-WASp–Arp2/3 pathway.** Confocal images of either WIP WT and KO or N-WASp WT and KO MEFs transfected with a combination of mCherry-actin, membrane-bound Nck SH3 fusion, and GFP-WIP or GFP–N-WASp demonstrate the necessity of WIP for Nck-induced, N-WASp–dependent actin polymerization. Higher magnifications of clusters are shown in the insets. Bars, 10 µm. (A) Antibody-induced aggregation of Nck SH3 domains (green) induces the formation of actin comet tails (red) in WIP WT MEFs (top) but does not induce actin polymerization in WIP KO MEFs (bottom). (B) Aggregation of Nck SH3 domains (cyan) in WIP KO MEFs rescued with GFP-WIP (green) induces actin comet tails (red) similar to those seen in WIP WT MEFs. (C) Nck SH3 aggregates (cyan) neither efficiently recruit emerald–N-WASp (green) nor induce actin polymerization (red) in WIP KO MEFs (top), whereas Nck SH3 aggregates in WIP WT MEFs both recruit emerald–N-WASp and induce actin polymerization (bottom). (D) Nck SH3 aggregates (cyan) recruit GFP-WIP (green) in both N-WASp KO MEFs (top) and N-WASp WT MEFs (bottom) but only induce actin polymerization (red) in N-WASp WT MEFs.

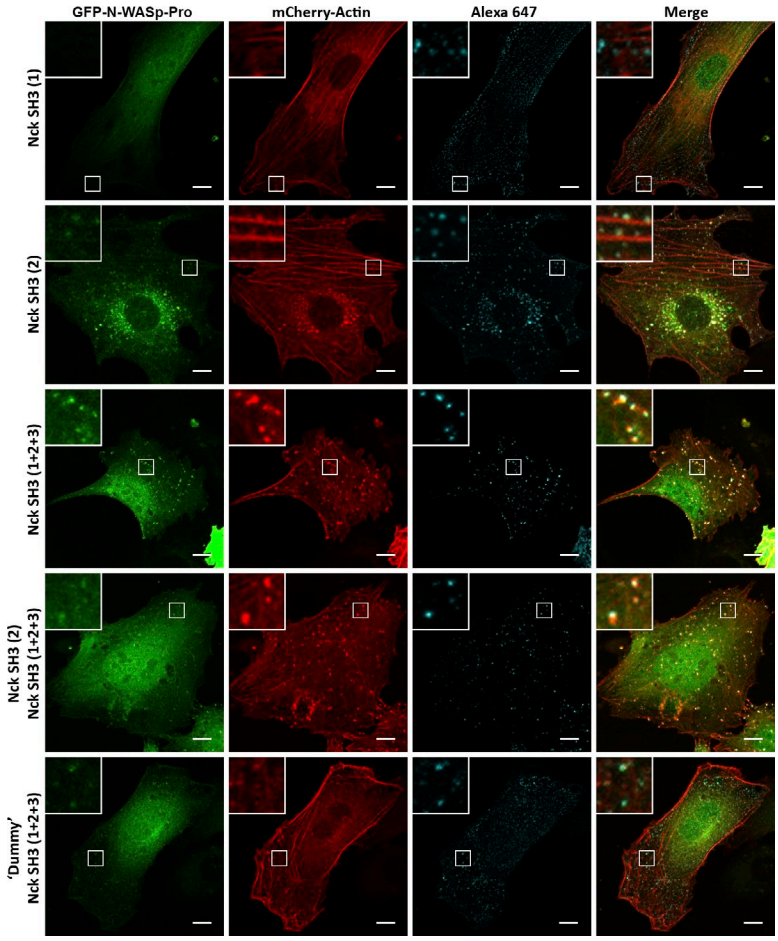

Figure S3. **Full activation of N-WASp requires both Nck SH3 (2) and Nck SH3 (1+2+3) in aggregates.** Confocal microscopy images of NIH-3T3 cells cotransfected with mCherry-actin, GFP–N-WASp-Pro (containing the proline-rich segment of N-WASp that binds Nck), and CD16-7–Nck SH3 (1) or CD16-7–Nck SH3 (2), CD16-7–Nck SH3 (1+2+3), CD16-7–dummy, or a combination of CD16 fusion proteins. For all images, mCherry-actin is red, GFP–N-WASp-Pro is green, and CD16 aggregates are cyan. Nck SH3 (1) aggregates (top) did not recruit GFP–N-WASp-Pro or induce actin polymerization. Nck SH3 (2) aggregates (second from top) and Nck SH3 (1+2+3)/dummy aggregates (bottom) recruited GFP–N-WASp-Pro and induced actin polymerization in the form of actin spots. Both Nck SH3 (1+2+3) (middle) and Nck SH3 (2)/Nck SH3 (1+2+3) (second from bottom) aggregates induce actin comet tail–like structures. Higher magnifications of clusters are shown in the insets. Bars, 10 µm.