Why Does Synergistic Activation of WASP, but Not N-WASP, by Cdc42 and PIP_2 Require Cdc42 Prenylation?

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Supplementary Information

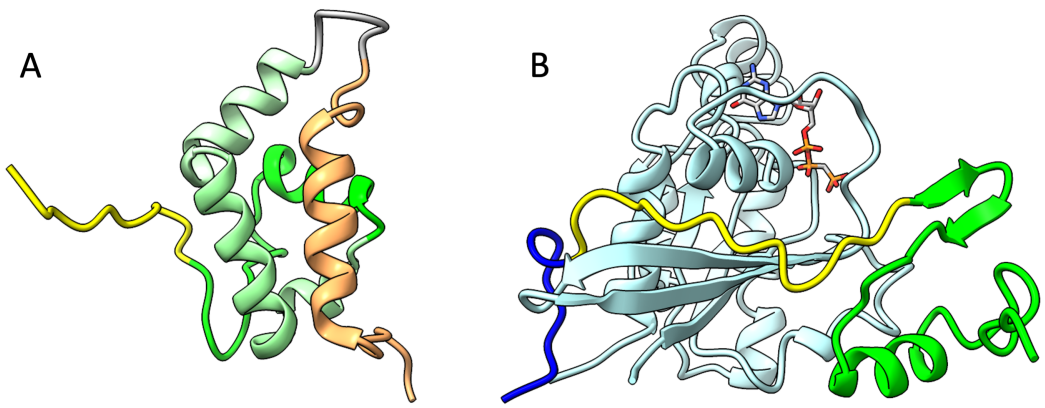

Figure S1. NMR structures of the WASP GBD in autoinhibited and activated states. (A) GBD bound with the C motif, from Protein Data Bank entry 1EJ5. CRIB and C motif are in yellow and orange; the GBD portion that binds to Cdc42 is in green, whereas the remaining portions are in two successively dimmer shades of green. (B) GBD bound to Cdc42. In addition to the colors for the regions present in (A), BR is in blue and Cdc42 is cyan; a GTP is shown as stick.

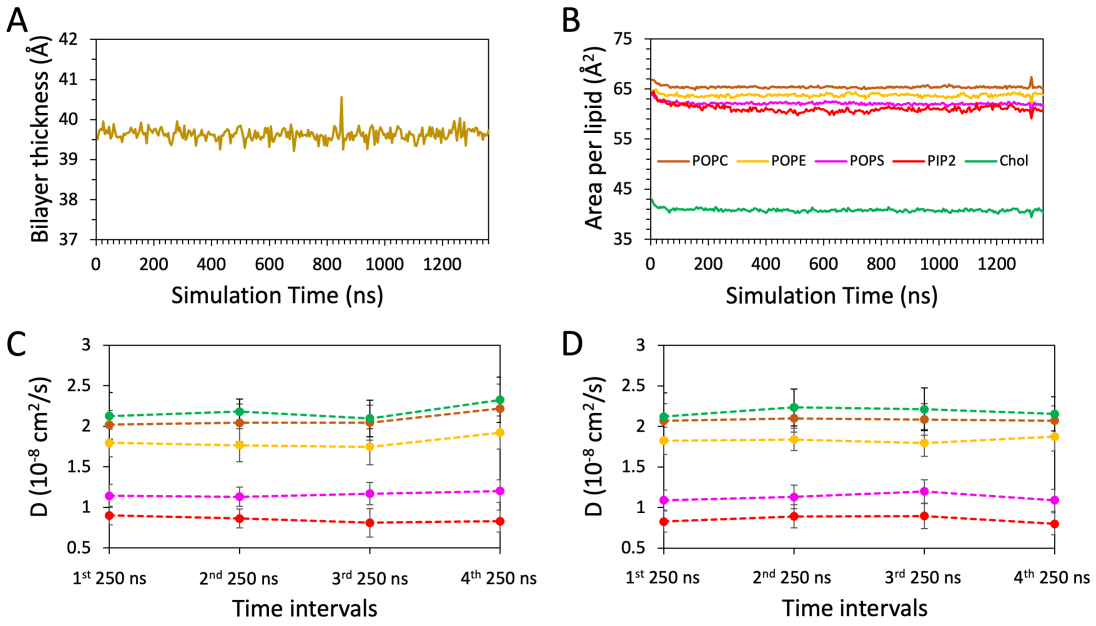

Figure S2. Membrane structural and dynamic properties in WASP and N-WASP simulations without Cdc42. (A) Time trace of the bilayer thickness, averaged over 16 WASP simulations. (B) Time trace of area per lipid for different lipids, averaged over 16 WASP simulations. (C) Lateral diffusion constants calculated in four 250-ns blocks after discarding the first 360 ns, shown as averages (circles) and standard deviations (error bars) calculated among 16 WASP simulations. The lag time ranged from 5 ns to 125 ns with 5 ns increments. (D) Corresponding results for N-WASP. The near constant values for each property across the simulation time indicate good equilibration of the lipids. Note that the lateral diffusion constants of the lipids match almost perfectly between WASP and N-WASP simulations, providing further support for equlibration.

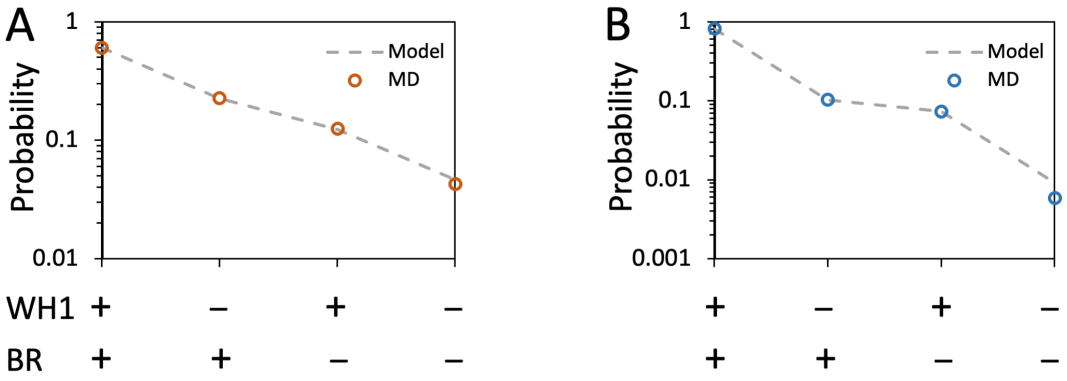

Figure S3. Comparison of WH1 and BR membrane association probabilities with the predictions assuming independence between the two regions in membrane association. (A) WASP. (B) N-WASP. The statistics obtained from MD simulations are shown as circles and model predictions are shown as dashed lines, for four possible states (bound and unbound denoted by + and – signs).

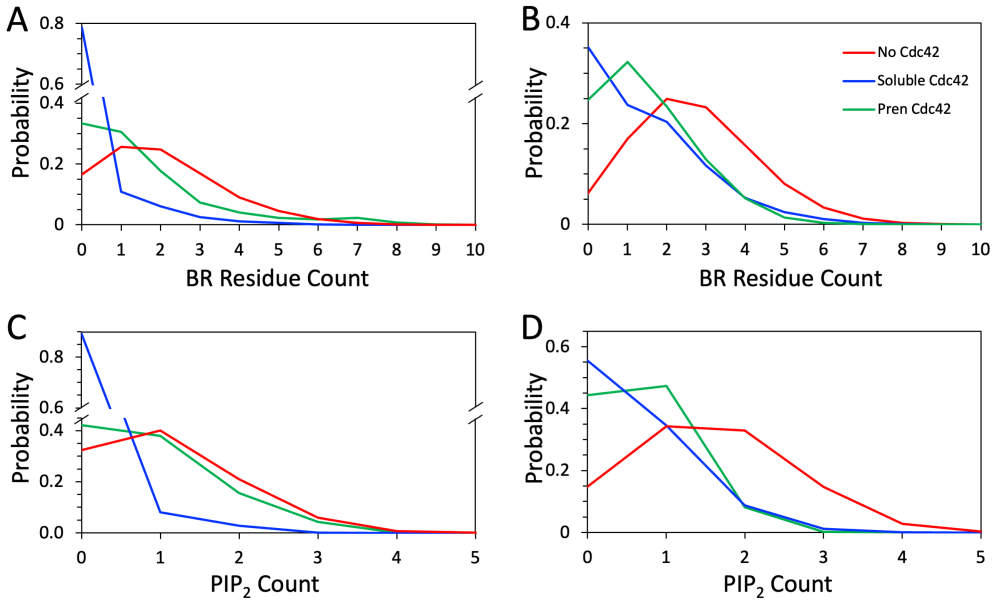

Figure S4. Probability distributions for *n* BR residues simultaneously bound to membranes and for *m* PIP_2 lipids simultaneously bound to the BR. (A) Distributions for *n* (BR residue count) in WASP simulations. (B) Corresponding plots for N-WASP. (C) Distributions for *m* (PIP_2 count) in WASP simulations. (D) Corresponding plots for N-WASP. Results from simulations without Cdc42, with soluble Cdc42, and with prenylated Cdc42 are in red, blue, and green, respectively.

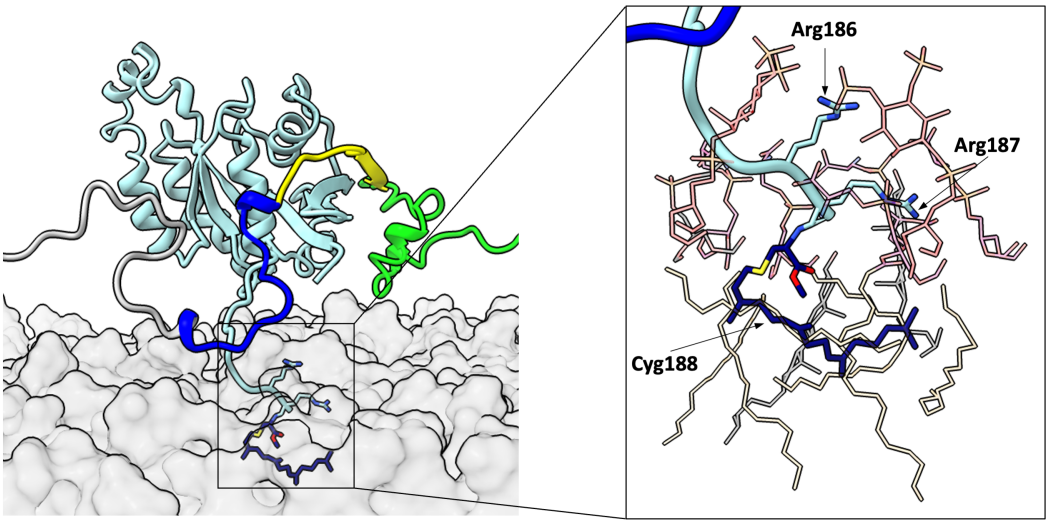

Figure S5. A representative snapshot from MD simulations of WASP prebound to prenylated Cdc42, showing the tethering of Cdc42 to the membrane by the prenyl group on the terminal Cys residue (Cyg188). In the normal view, lipids are shown as gray surface and the sidechains of the terminal three residues are shown as stick. In the zoomed view, lipids that are in contact with the terminal three residues are also shown as stick, in color for headgroups and in gray for tails. The prenyl group forms extensive nonpolar interactions with lipid tails while Arg186 and Arg187 form electrostatic interactions with lipid headgroups.

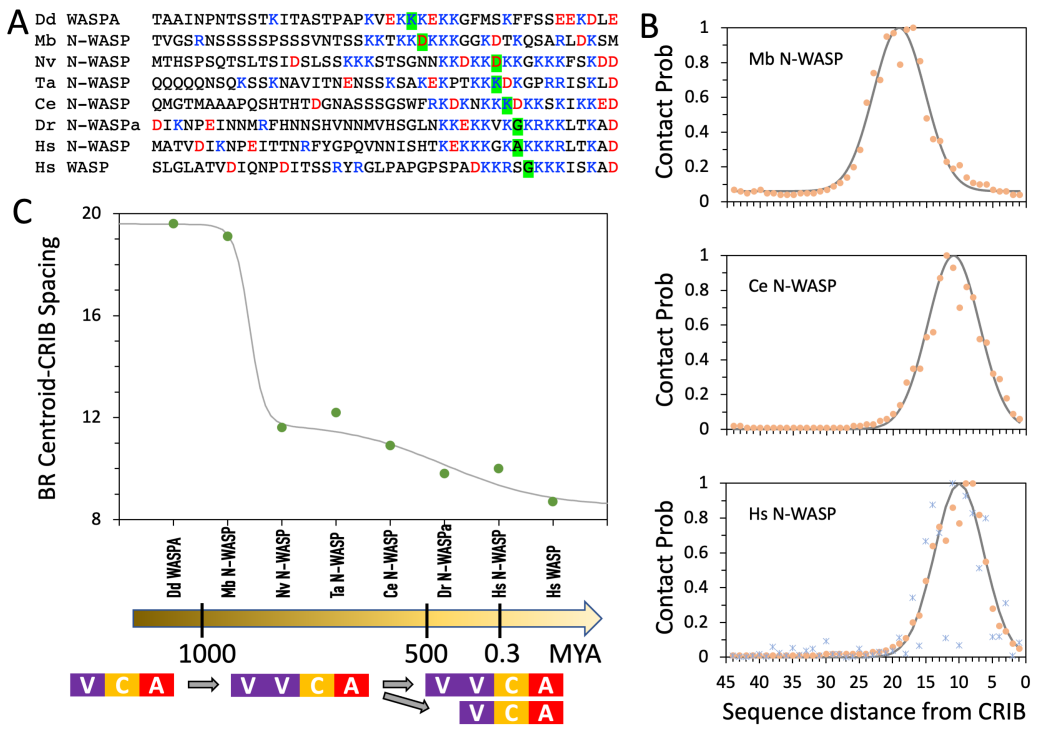

Figure S6. Spacing between BR centroid and CRIB in WASP orthologs. (A) Sequence comparison for 44 amino acids immediately before CRIB in eight WASP orthologs. The amino acid closest to the BR centroid identified in (B) is shaded in green. Abbreviations (and UniProt entry names) are: Dd, *Dictyostelium discoideum* (Q9GSG9); Mb, *Monosiga brevicollis* (A9V6R1); Nv, *Nematostella vectensis* (A7RXK9); Ta, *Trichoplax adhaerens* (B3RTH0); Ce, *Caenorhabditis elegans* (Q8MQE6); Dr, *Danio rerio* (F1QNY1); Hs, *Homo sapiens* (O00401 for N-WASP and P42768 for WASP). (B) Membrane association propensities predicted by ReSMAP [30] from selected sequences in (A), shown as orange circles; Gaussian fits are shown as solid curves. The peak position of each Gaussian is identified as the BR centroid. For Hs N-WASP, the membrane contact probabilities from the MD simulations, scaled to a maximum of 1, are shown as blue asterisks. (C) The progression of the BR centroid-CRIB spacing over evolutionary time (million years ago, or MYA). The curve is only for guiding the eye. Invertebrates emerged shortly after 1000 MYA; vertebrates emerged ~500 MYA; humans emerged 0.3 MYA. The classification into N-WASP is based on the presence of an extra V motif. According to Veltman and Insall [12], metazoan N-WASPs descended from ancestral WASPs, whereas vertebrate WASPs branched off from this lineage.