EFC/F-BAR proteins and the N-WASP-WIP complex bind to membranes to induce membrane-curvature dependent actin polymerization

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Supplementary information

Figure Legends

Figure S1. Illustration of the domain structures of representative proteins used in this study.

Figure S2. (**A**) Binding of wild-type (WT) N-WASP, Δbasic N-WASP, N-WASP-WIP complex, and WIP to GST-fusion proteins of the SH3 domain of FBP17 or amphiphysin was examined with a pull-down assay. The N-WASP, WIP, or N-WASP-WIP concentration is indicated. (**B**) Binding of wild-type (WT) N-WASP, N-WASP-WIP complex, and WIP to GST-fusion proteins of full length of Toca-1 was examined with a pull-down assay.

Figure S3. Transmission electron microscopy of negatively stained Folch liposomes: LMVs, SUVs, and liposomes extruded through cyclopore filters with pore sizes of 400 nm, 100 nm, and 50 nm.

Figure S4. The myc- and GFP-N-WASP-FLAG-WIP complex proteins and GST-Toca-1 or FBP17 proteins used in this study were visualized with Coomassie brilliant blue staining after SDS-PAGE.

Figure S5. Transmission electron microscopy of negatively stained 0.1 mg/ml LMVs in the presence of 600 nM (**A**) GST-EFC/F-BAR domain protein, (**B**) wild type (WT) of GST-FBP17, or (**C**) HHK mutant of GST-FBP17. (**D**) Distribution of the diameters of the tubules in **A-C**.

Fig. S6. Quantification of binding of (**A**) WIP, (**B**) N-WASP, and (**C**) N-WASP-WIP complex to phosphoinositides. Binding of 400 nM of each protein to 1 mg/ml liposomes (PE/PC/PIPx = 8/2/1, PE/PC/PI, PE/PC/PS, or PE/PC/PA = 8/2/3) was examined. Binding of the N-WASP-WIP complex to liposomes was measured by N-WASP binding. Error bars indicate standard deviation (S.D.).

Figure S7. Induction of membrane curvature-dependent actin polymerization by N-WASP. (**A**) Fold activation of actin polymerization by 15 nM N-WASP alone or N-WASP-WIP complex and 20 μg/ml Folch LMVs or SUVs. (**B**) Actin polymerization induced by 150 nM N-WASP, 600 nM GST-FBP17, and/or 20 μg/ml Folch LMVs. (**C**) Fold activation of actin polymerization by 600 nM GST-FBP17 in the presence of 300 nM N-WASP alone with 20 μg/ml Folch SUVs or LMVs. (**D**) Actin polymerization induced by 300 nM Δbasic mutant N-WASP, 600 nM GST-FBP17, and/or 20 μg/ml Folch LMVs. (**E**) Actin polymerization induced by 30 nM N-WASP, 4 μM GST-Cdc42 (GTPγS-loaded), 600 nM GST-Toca-1, and/or 20 μg/ml PIP2/PC/PE LMVs (PE/PC/PIP_2 = 8/2/1). (**F**) Actin polymerization induced by 30 nM N-WASP, 4 μM GST-Cdc42 (GTPγS-loaded), and/or 20 μg/ml Folch LMVs.

Figure S8. Cdc42-mediated acceleration of Toca-1-induced activation of the N-WASP-WIP complex. (**A, B**) Actin polymerization induced by 150 nM N-WASP, 600 nM GST-Toca-1, 2 μM Cdc42 (GTPγS-loaded), and/or 20 μg/ml Folch LMVs (**A**) or SUVs (**B**). (**C**) Actin polymerization induced by 30 nM N-WASP, 4 μM GST-Cdc42 (GTPγS-loaded), 600 nM wild-type (WT) or IST mutant of GST-Toca-1, and/or 20 μg/ml Folch LMVs.

Figure S9. Recruitment of N-WASP or N-WASP-WIP complex to the membrane by the

HHK mutant of FBP17. We incubated 300 nM N-WASP-WIP complex, 400 nM wild-type (WT) N-WASP, 400 nM Δbasic N-WASP, or 300 nM WIP with 600 nM wild type or HHK mutant of GST-FBP17 and 50 μg/ml Folch LMVs. Proteins bound to LMVs were examined by co-sedimentation assay.

Figure S10. Recruitment of WIP to the membrane by the HHK mutant of FBP17. We incubated 300 nM WIP with 600 nM wild type or HHK mutant of GST-FBP17 and 50 μg/ml Folch LMVs. Proteins bound to LMVs were examined by co-sedimentation assay.

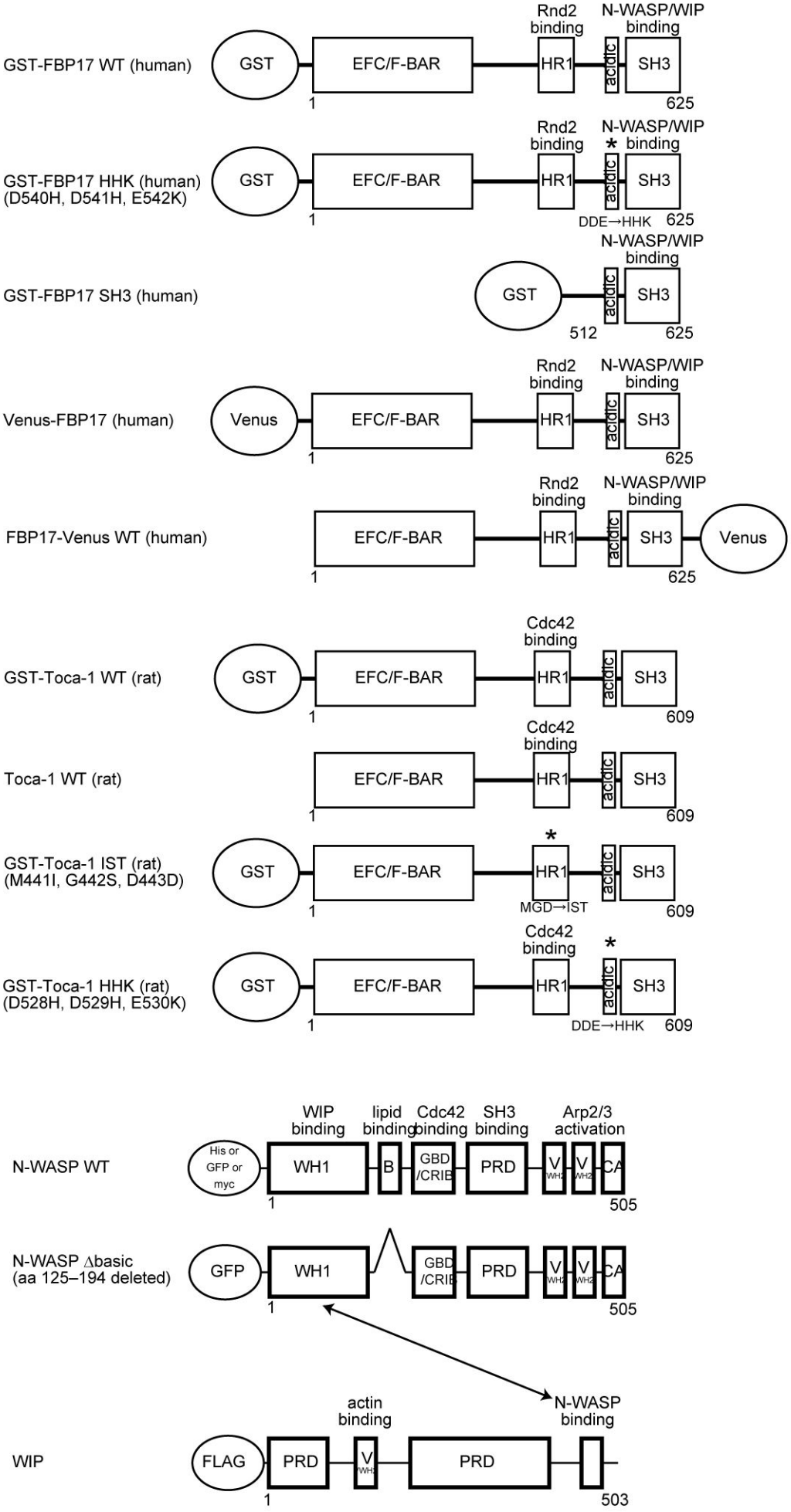


Figure S1, Suetsugu et al.

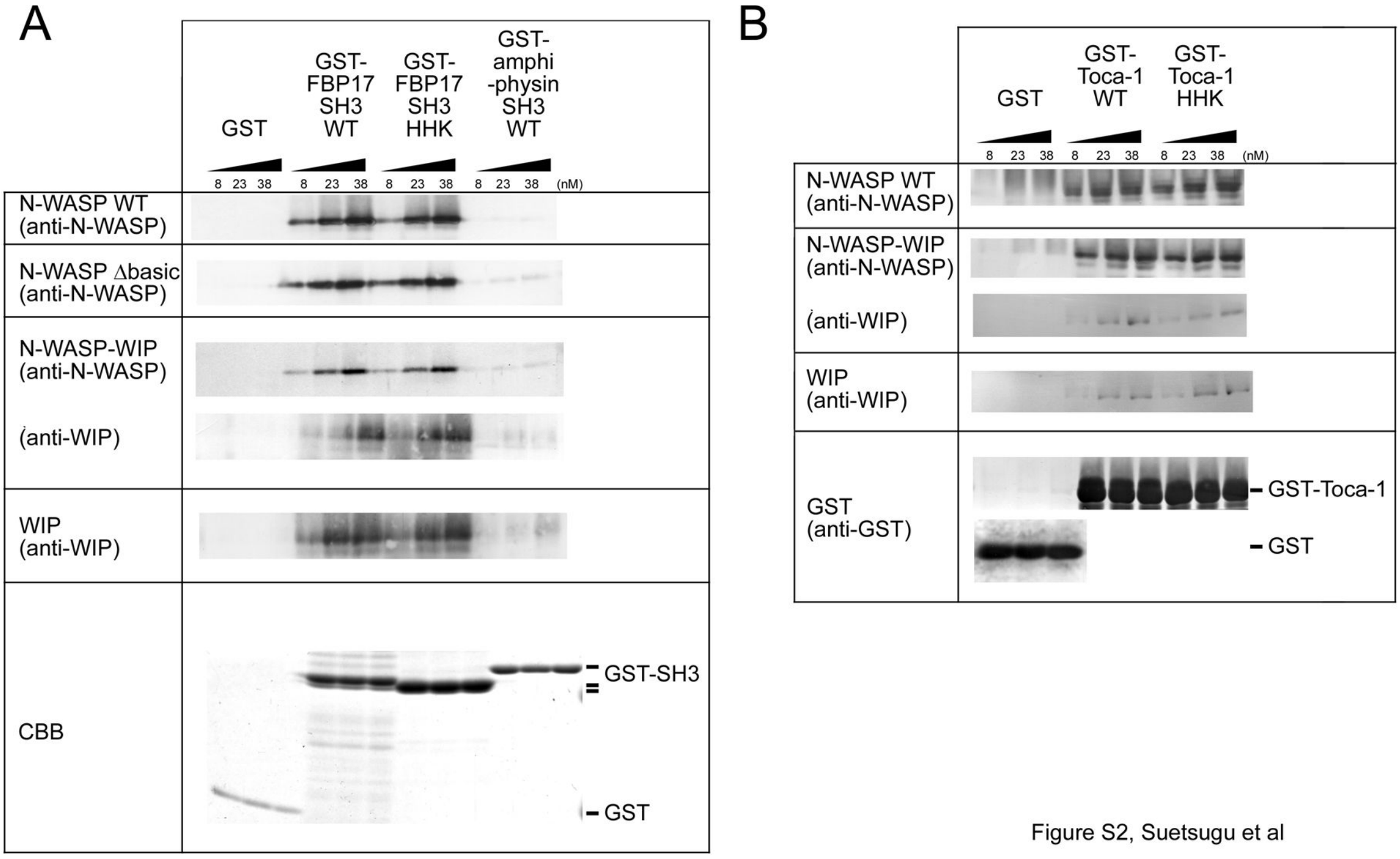


Figure S2, Suetsugu et al

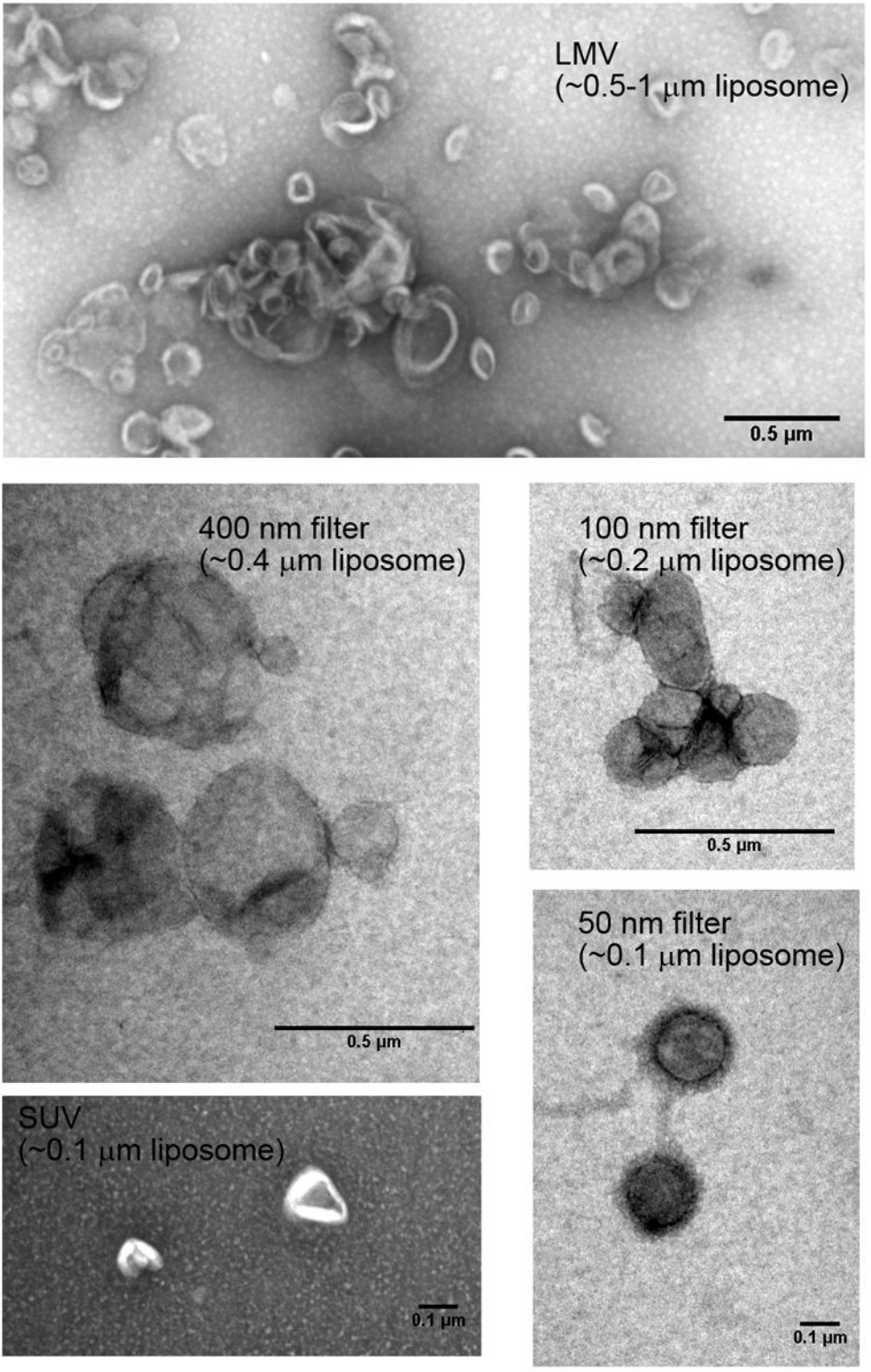
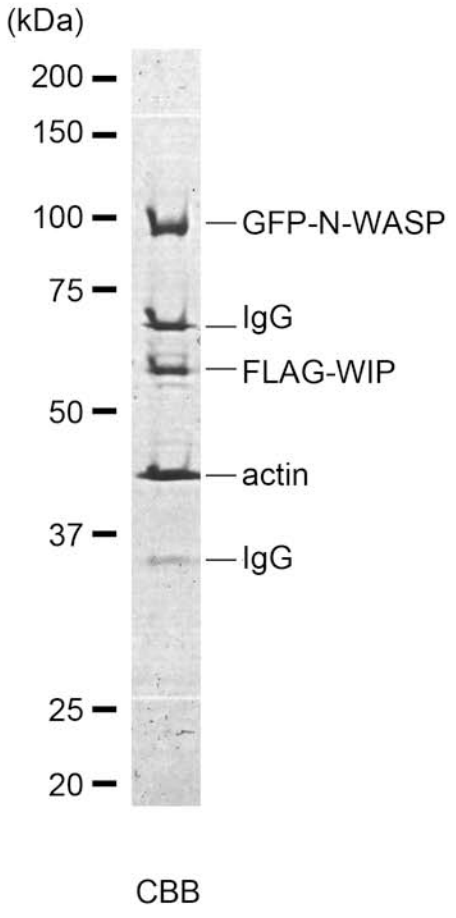
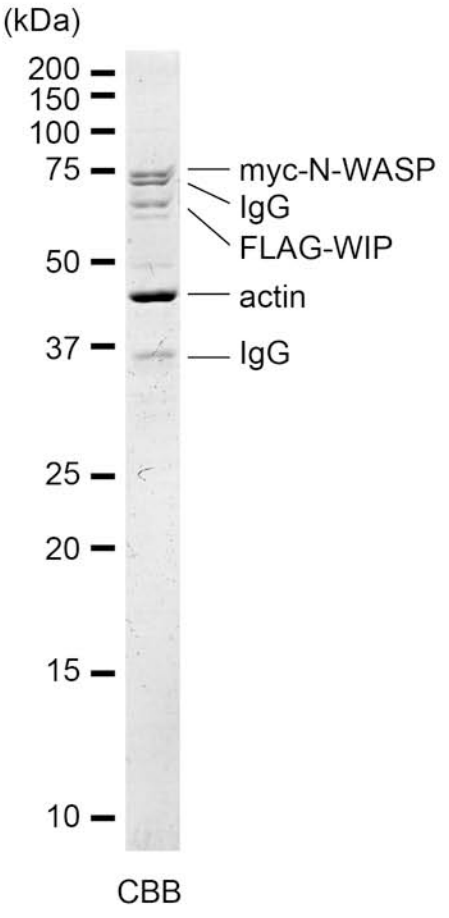


Figure S3, Suetsugu et al





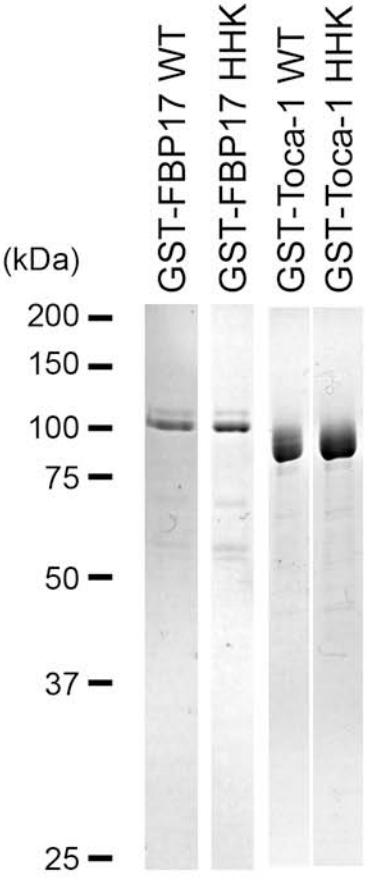
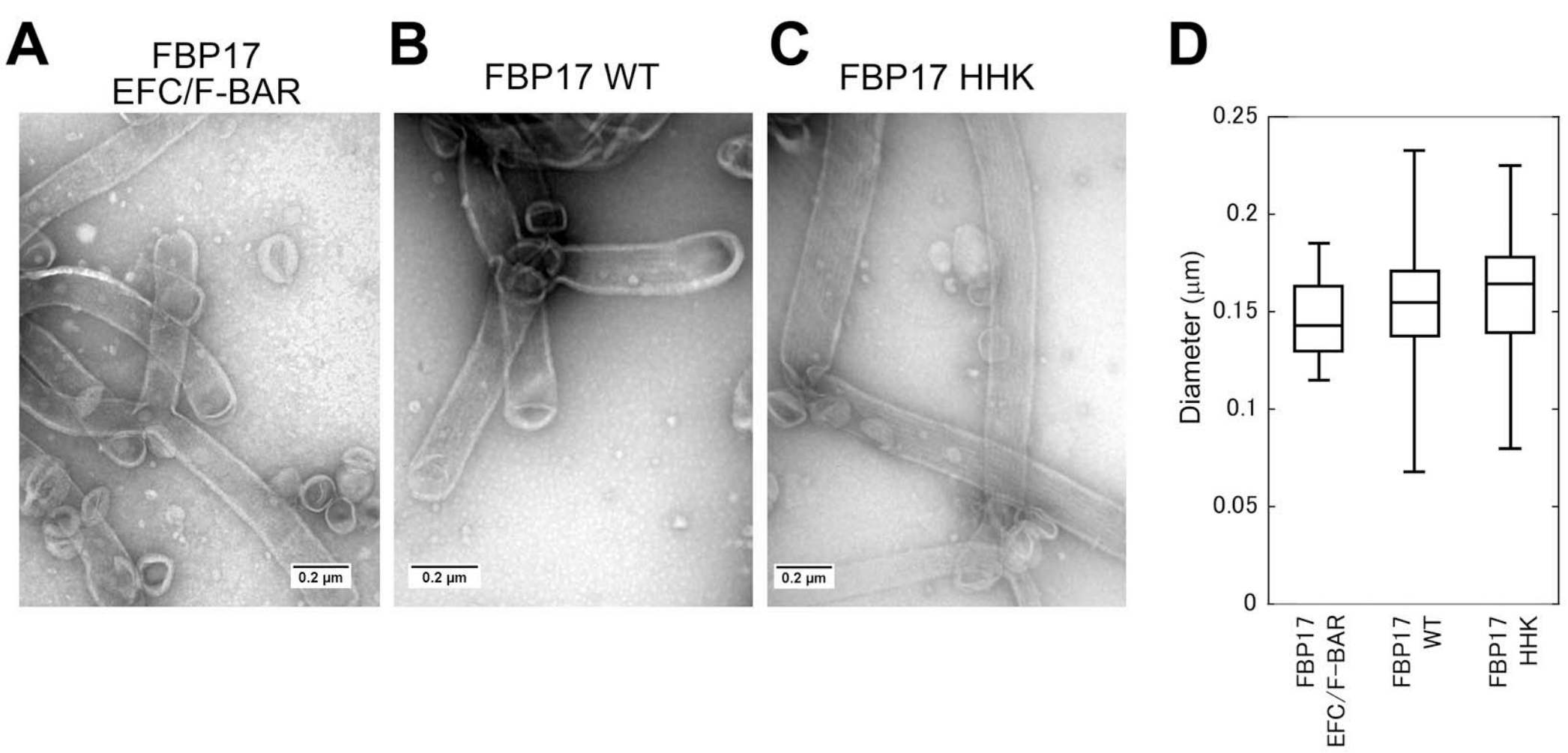


Figure S4, Suetsugu et al



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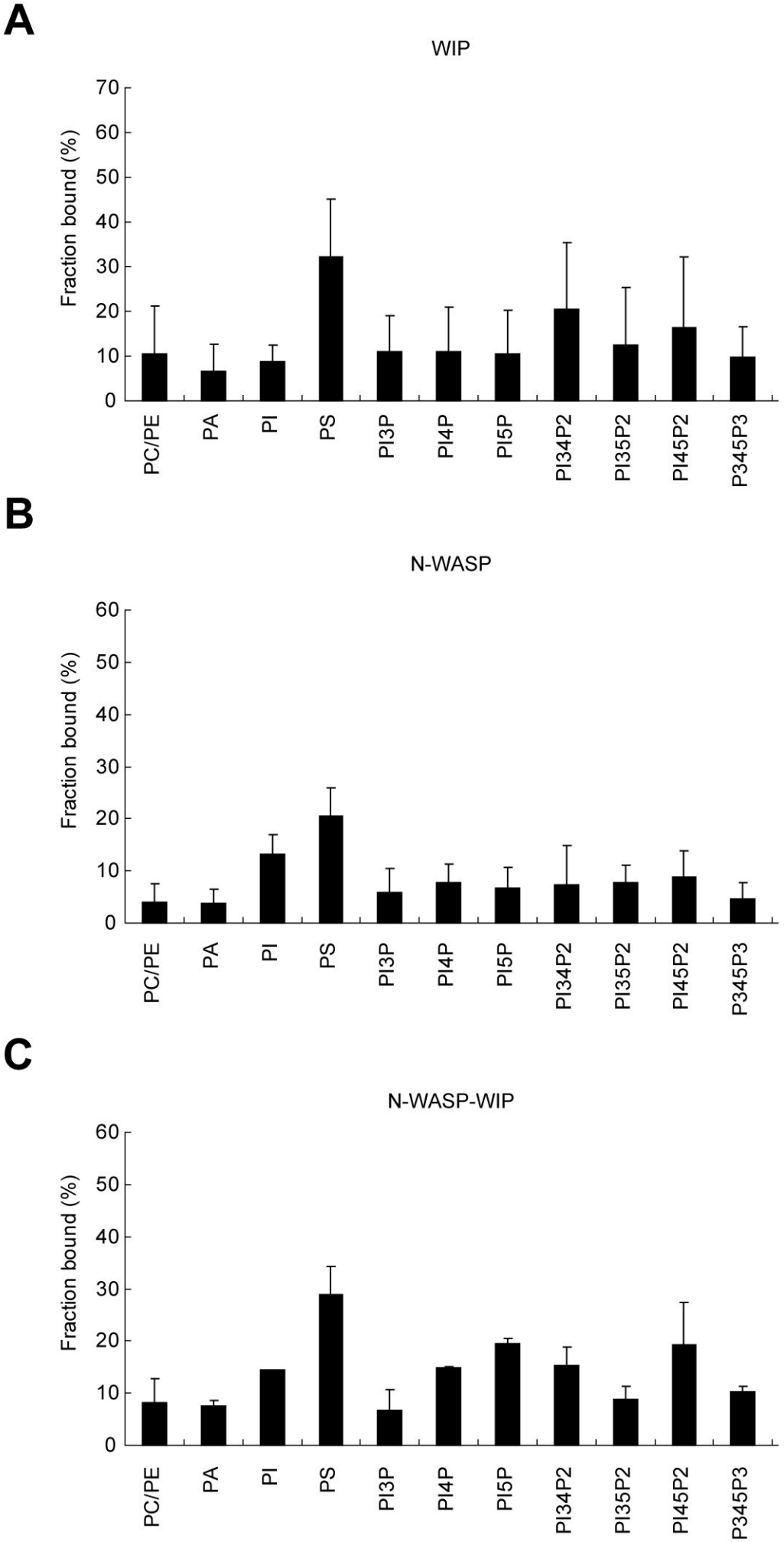

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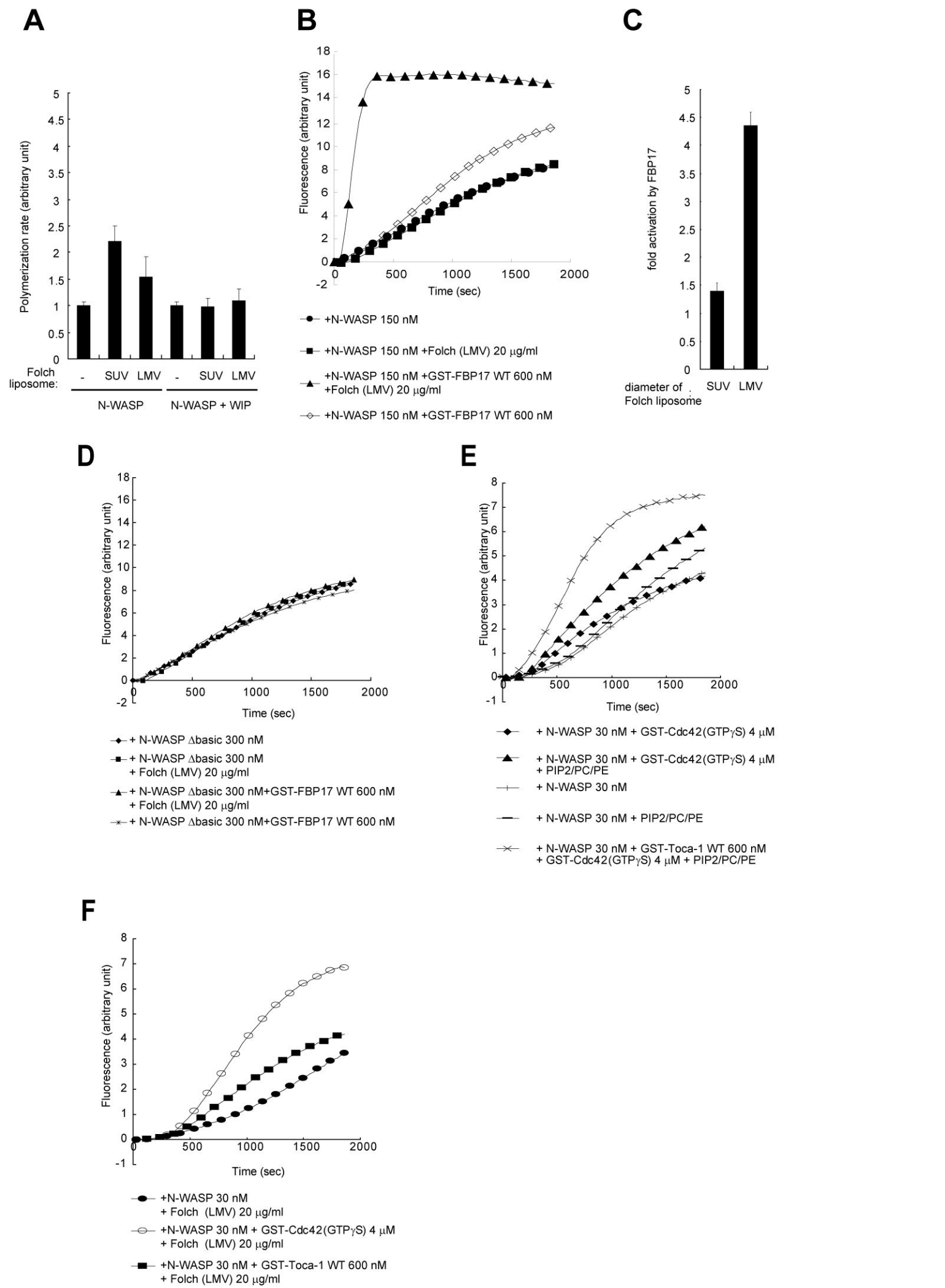

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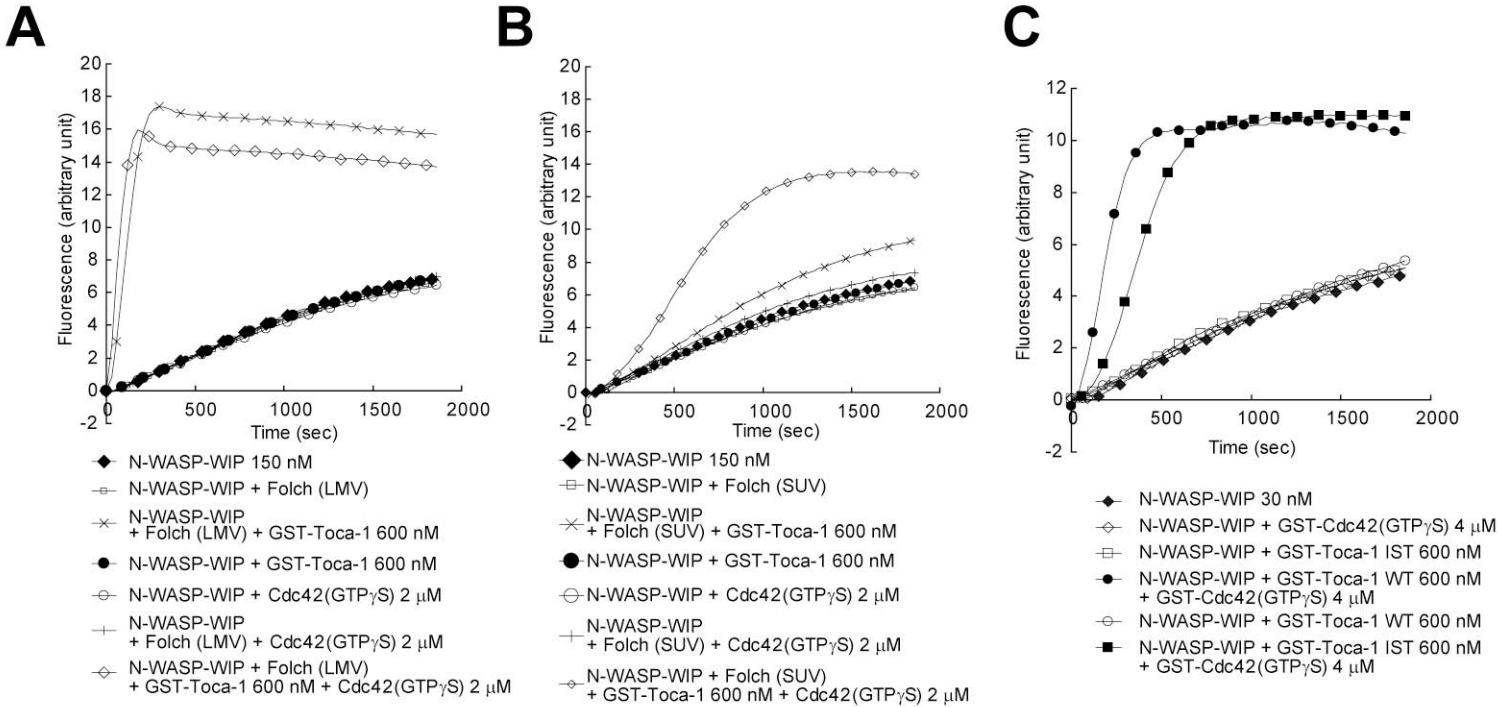

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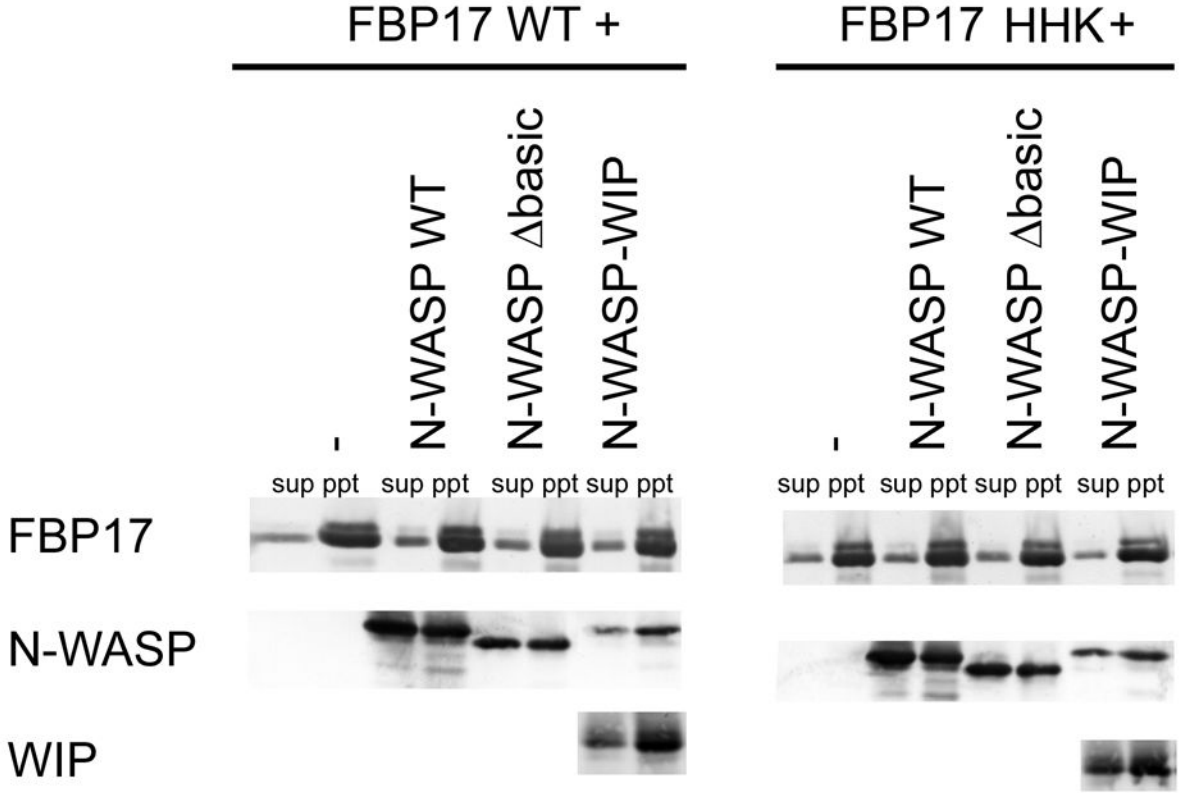

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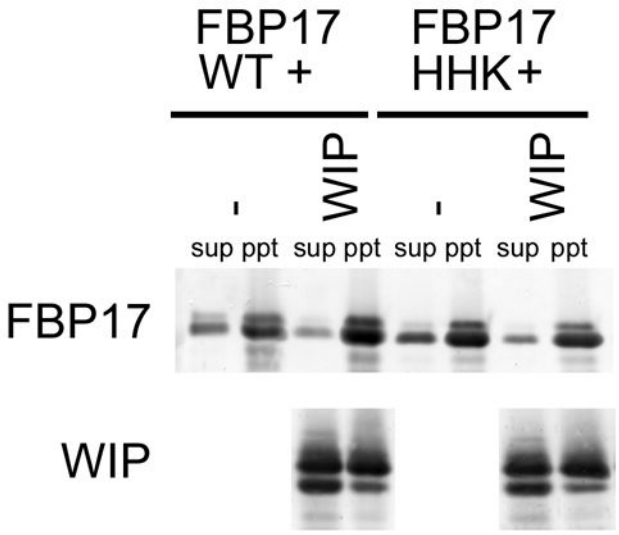

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