

Fig. S1. Conservation and alignments of the srGAP family of proteins. (A-B) Phylogenetic tree illustrating the evolutionary conservation of human full-length srGAP1 (NP_065813.1), srGAP2 (NP_056141.2), srGAP3 (NP_001028289.1), and srGAP4/ArhGAP4 (NP_001158213.1) (A), and F-BAR(1) (AA 1-516), F-BAR(2) (AA 1-501), F-BAR(3) (AA 1-492), and F-BAR(4) (AA 1-557). (B). (C) Domain structure of human full-length srGAP1, srGAP2, and srGAP3. Percentages indicate conserved amino acids in the sequence of each domain of srGAP1 and srGAP3, compared to the same domain in srGAP2. (D) Sequence alignment of F-BARs (1-3). Red asterisks point out a sequence specific insertion (AA 197-207) in srGAP2, and red carets denote an insertion (AA493-516) in srGAP1. (E-F) Expression of srGAP1-myc, srGAP-GFP, and srGAP3-6xmyc fusion proteins (E) and F-BAR-GFP fusion proteins (F) to illustrate proper expression and detection of srGAP proteins. The commercially available anti-srGAP1 antibody recognizes all three full-length proteins, and therefore was not used for the remainder of the study. Untagged srGAP protein bands appear at the same molecular weight in Western blot (data not shown). (G) srGAP2 levels in E15.5 cortical neurons cultured for 2 and 4 days in vitro.

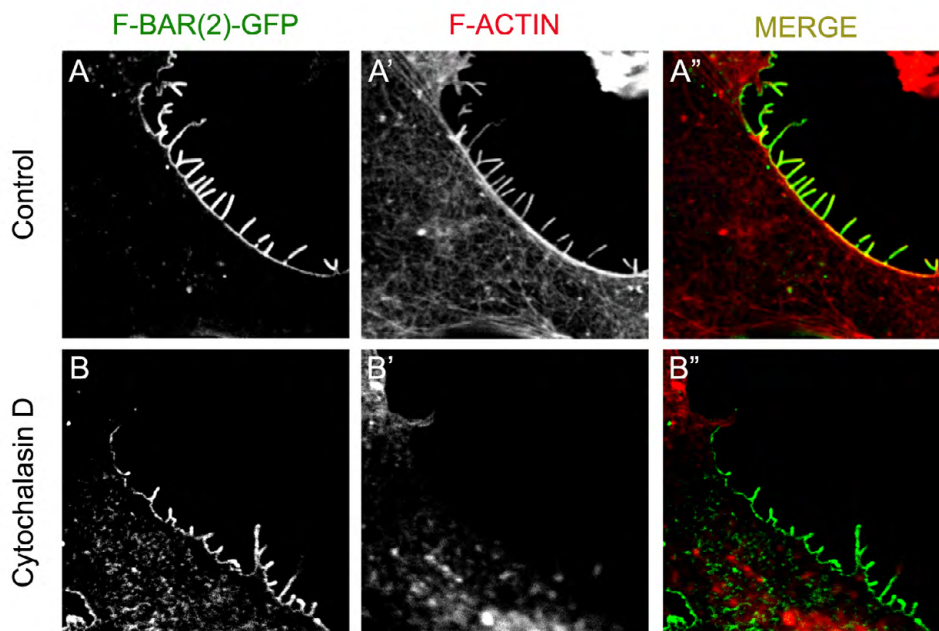


Fig. S2. The F-Actin Network is disrupted after Cytochalasin-D treatment. (A-A'') Control COS7 cell used for FRAP experiments. This cell, transfected with F-BAR(2), displays a normal, intact F-actin network when stained with phalloidin. **(B-B'')** Validation of F-actin depolymerization after cytochalasin-D treatment in a COS7 cell used in FRAP experiments.

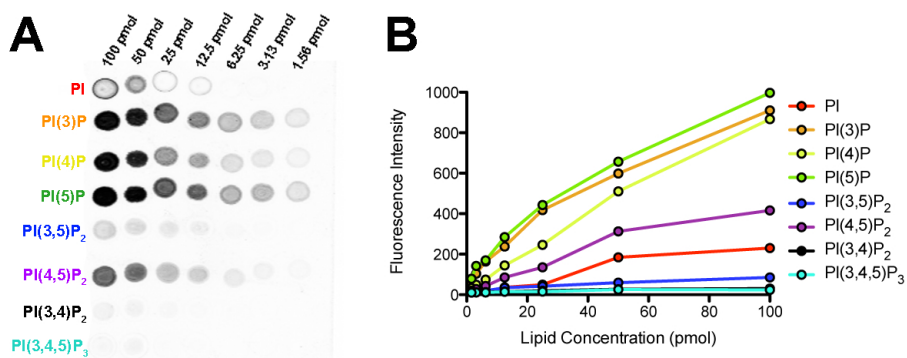


Fig. S3. Dose-dependent binding of F-BAR(2) to various phosphoinositides. (A) PIP array showing lipid-binding specificity of recombinant F-BAR(2) for different concentrations of phosphorylated phosphatidylinositol (PI). **(B)** Quantification reveals that F-BAR(2) strongly binds to singly-phosphorylated species of PI, has intermediate association with PI(4,5)P₂ and PI, and low affinity for PI(3,5)P₂, PI(3,4)P₂, and PI(3,4,5)P₃.

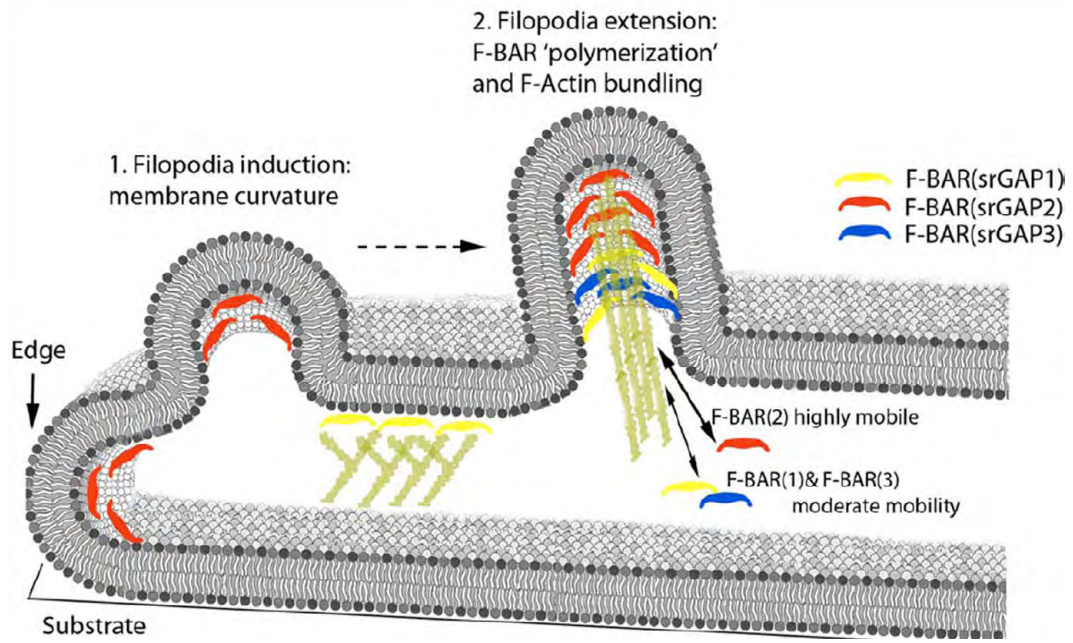


Fig. S4. Model of the functional properties of the F-BAR domains of three srGAP family members. See text for details.