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# Supplemental Information

# Structural Insights into Assembly and Regulation of the Plasma Membrane Phosphatidylinositol 4-Kinase Complex

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## Inventory of Supplemental Materials

**Fig. S1**. This figure is related to Table 1. It shows the quality of electron density maps into which Efr3 and Ypp1 were modeled, as well as of the final maps after refinement.

**Fig. S2**. This figure is related to Fig. 1 and 3. Efr3 and Ypp1 constructs similar to those used in the *in vivo* studies were recombinantly expressed, and soluble proteins were purified and characterized by circular dichroism to demonstrate folding. All the constructs were soluble and well folded, except for Efr3-4, which aggregated, and Ypp1$_{11\text{-}805\Delta 806\text{-}817}$, which was not well solubilized.

**Fig. S3** is related to Fig. 2. Liposome binding assays were carried out using the flotation rather than sedimentation method. The results are similar.

**Fig. S4** is related to Fig. 3 and shows the superposition of 8 crystallographically independent copies of Ypp1, illustrating flexibility in the Ypp1 structure.

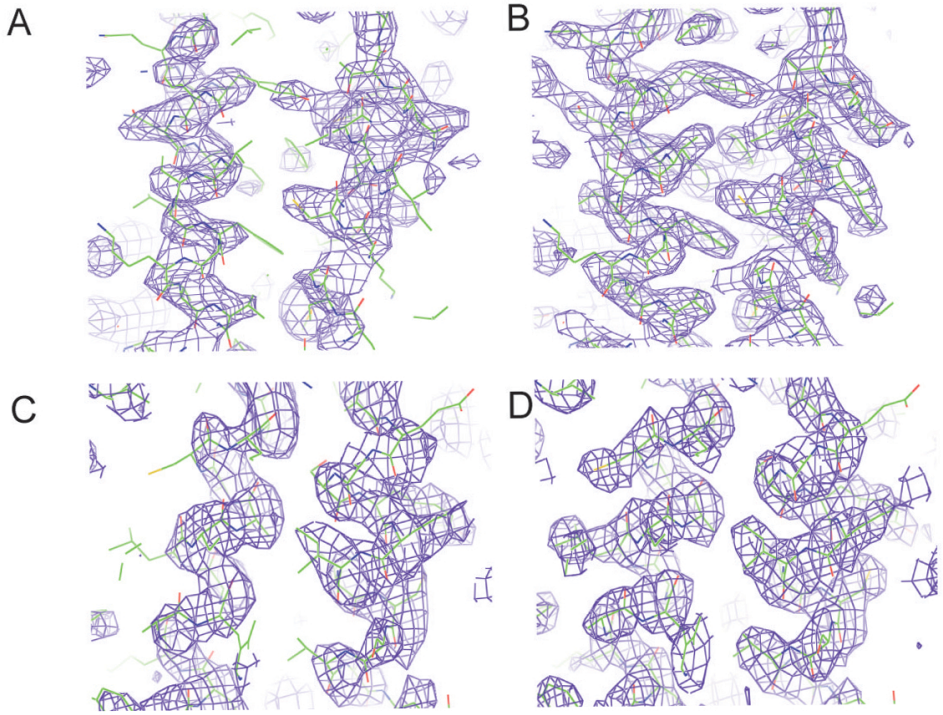


**Fig. S1 (related to Table 1)**. Electron density maps. (A) Map, contoured at 1.5σ, into which Efr3 was modeled. This map was calculated by combining SAD phases with phases from a partial model of Efr3 (not including portions shown), and sharpening B-factors (-50 Å$^2$). Final refined coordinates for Efr3 are superimposed in green. (B) The same region in a 2Fo-Fc map contoured at 1.5σ with B-factor sharpening (-50 Å$^2$). (C) Map, contoured at 1.5σ, into which Ypp1 was modeled. This map was calculated by combining SAD phases with phases calculated from Ypp1-N, averaging using NCS, and sharpening B-factors (-50 Å$^2$). Final refined coordinates for Ypp1 are superimposed. The region shown is in the C-terminal half of Ypp1 and was not used in the phase combination. (D) The same region in a 2Fo-Fc map contoured at 1.5σ with B-factor sharpening (-50 Å$^2$).

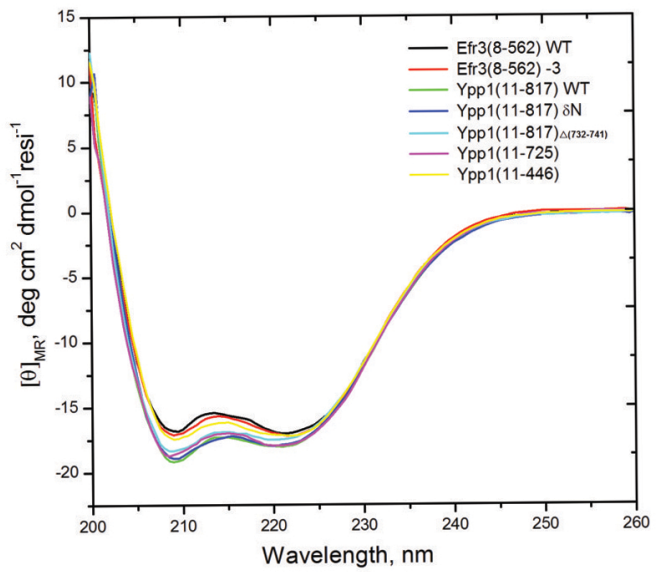


**Fig. S2 (related to Fig. 1)**. CD spectra for Efr3 and Ypp1 constructs, as indicated [Mean residue ellipticity (y-axis) vs wavelength (x-axis)]. For Efr3 constructs, measurements were carried out in 20mM Tris pH 8.0 (at 21°C), 150mM NaCl, 0.5mM TCEP, 10% glycerol at 22C. The buffer for Ypp1 constructs was the same but contained no glycerol. The concentration of protein in the assay is ~0.2-0.3 mg/ml. The proteins are well-folded and primarily alpha helical in their secondary structure.

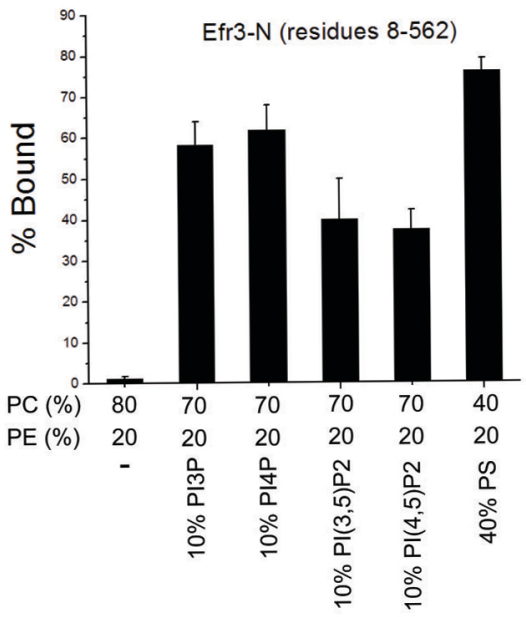


**Fig. S3 (related to Fig. 2).** Efr3-N binds to acidic membranes. These results are from a flotation assay; the experiment was carried out in duplicate. Error bars represent standard deviation.

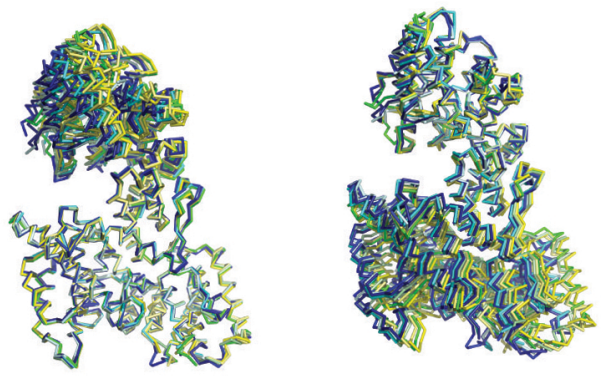

**Fig. S4 (related to Fig. 3)**. Superposition of the eight crystallographically independent copies of Ypp1, aligning residues 11-500 (left) or 501-817 (right), to demonstrate flexibility in Ypp1.