

Figure S4

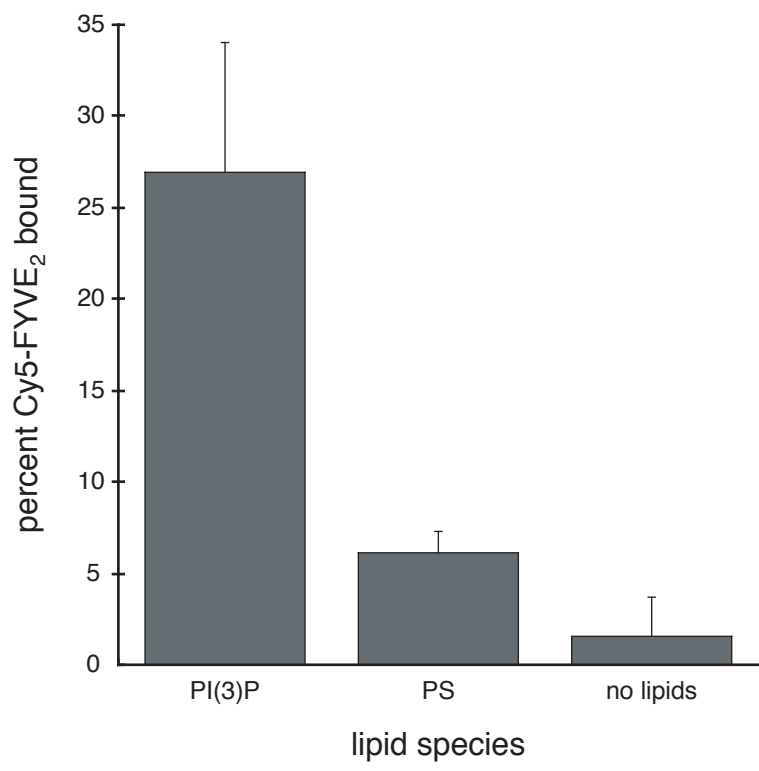


Figure S4 PI(3)P-containing liposomes bind a tandem FYVE domain construct. PS- or PI(3)P-containing liposomes (20 μ l; 3.2 mM final total lipid concentration) made as described in Figure 6B, or RB alone, were incubated with a FYVE₂ construct (Gillooly et al., 2000) that had been labelled with Cy5-maleimide (GE), and 20 μ l of the 1.8 M sucrose solution described in Figure 6B. After a 30 min. ice incubation, sucrose gradients were poured and the gradients were centrifuged as described in Figure 6B. Liposomes (40 μ l) were harvested, and recovered lipids and Cy5-FYVE₂ were quantified by fluorimetry ($\lambda_{\text{ex}}/\lambda_{\text{em}}$ 510/580 nm for Rh-DHPE, 630/680 nm for Cy5-FYVE₂); reported Cy5-FYVE₂ recovery was corrected for lipid recovery. Means and standard deviations for 3 independent experiments are shown.