**Fig. S2.** Comparisons of the human EAP45 and yeast Vps36p GLUE domains. (A) Superposition of human EAP45 (turquoise) and yeast Vps36p (pink) GLUE domains. (B) Expanded view of the Ub I44:EAP45 GLUE binding interface, with yeast Vps36p GLUE (pink) superimposed on EAP45 GLUE (turquoise) to illustrate differences between the EAP45 surface, which binds ubiquitin, and the Vps36p surface which apparently does not. Key interface residues are shown explicitly for Ub (green) and EAP45 GLUE (blue), and the equivalent residues in Vps36p GLUE are shown in magenta. Several key EAP45 GLUE Ub binding residues are not conserved in yeast Vps36p GLUE: EAP45 V67: Vps36p A75, EAP45 F68: Vps36p Y76, EAP45 E70: Vps36p N78 and that buttressing residues in the EAP45 GLUE S6/S7 loop (V83, H87, P88) are apparently missing owing to the larger insertion in the Vps36p protein. Note also that the EAP45 GLUE V67A mutation alone reduced Ub binding affinity more than 10-fold (Fig. 1 and Supplemental Table 2 online). (C) Expanded view showing the approximate locations of the canonical (PIA, orange) and non-canonical (PIB, dark magenta) phosphatidylinositol binding sites in the superimposed EAP45 GLUE and yVps36p GLUE domains. The PI molecules were positioned as described in Fig. 1C, and the color coding is the same as in panels A and B. Residues in Vps36p GLUE required for full affinity phospholipid binding are shown in magenta, additional residues in the apparent PI binding site are shown in pink, the position of a bound sulfate ion is shown in tan, and residues that can be mutated without loss of PI binding are shown in straw. Key residues in the apparent PI binding site of EAP45 GLUE are shown in blue. Note that a series of residues surrounding the non-canonical (PIB) site are conserved as basic residues in both the yeast and human proteins: EAP45 K32: Vps36p K38, EAP45 K78: Vps36p R86, EAP45 K81: Vps36p R89, EAP45 K111: Vps36p R261. The human protein also has three additional basic residues in this region (R25, K35, K107) that may account for the apparent preference of the human protein for more highly phosphorylated forms of 3-phosphoinositides such as P(3,4,5)P3.

**References**
