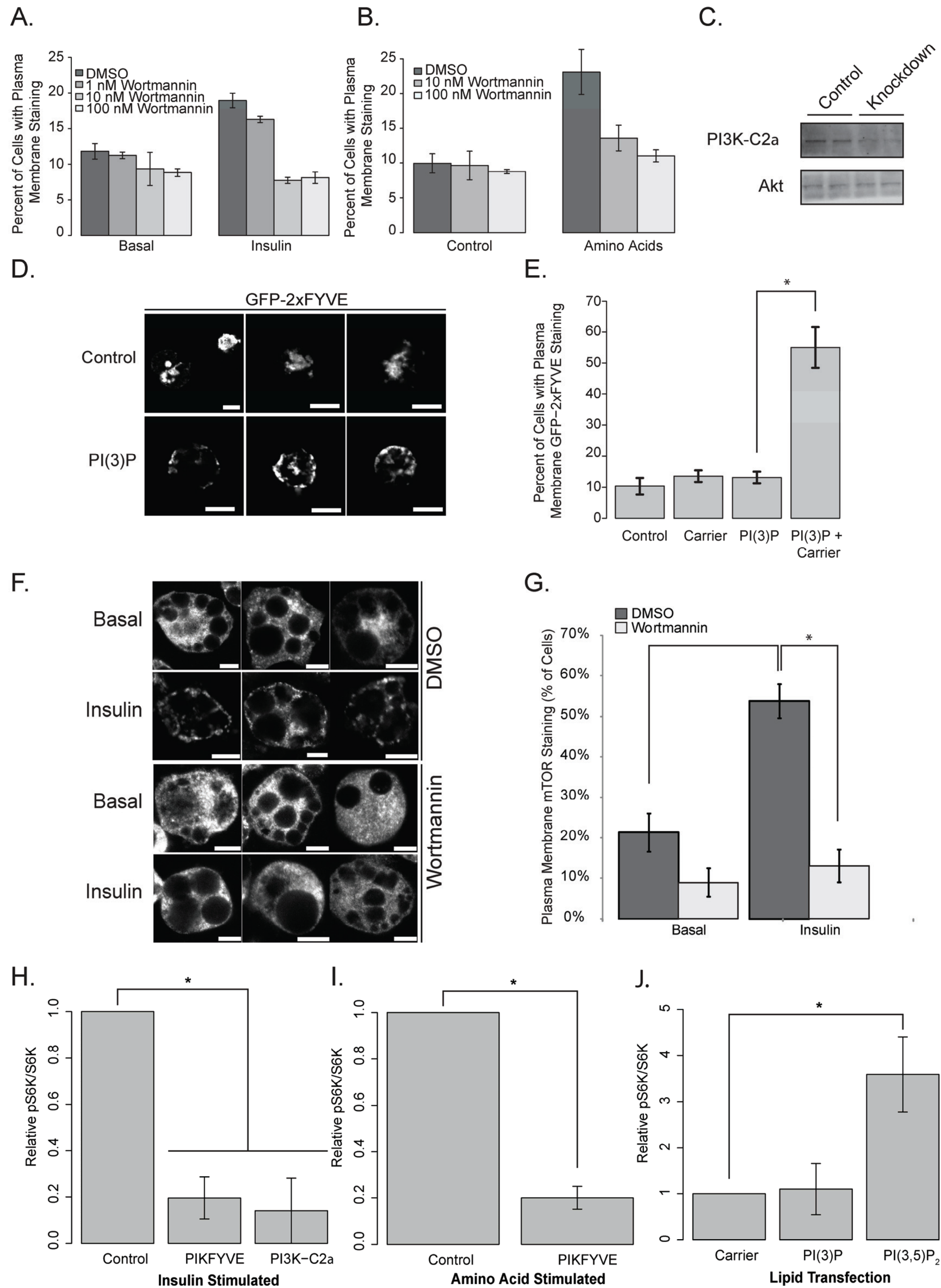
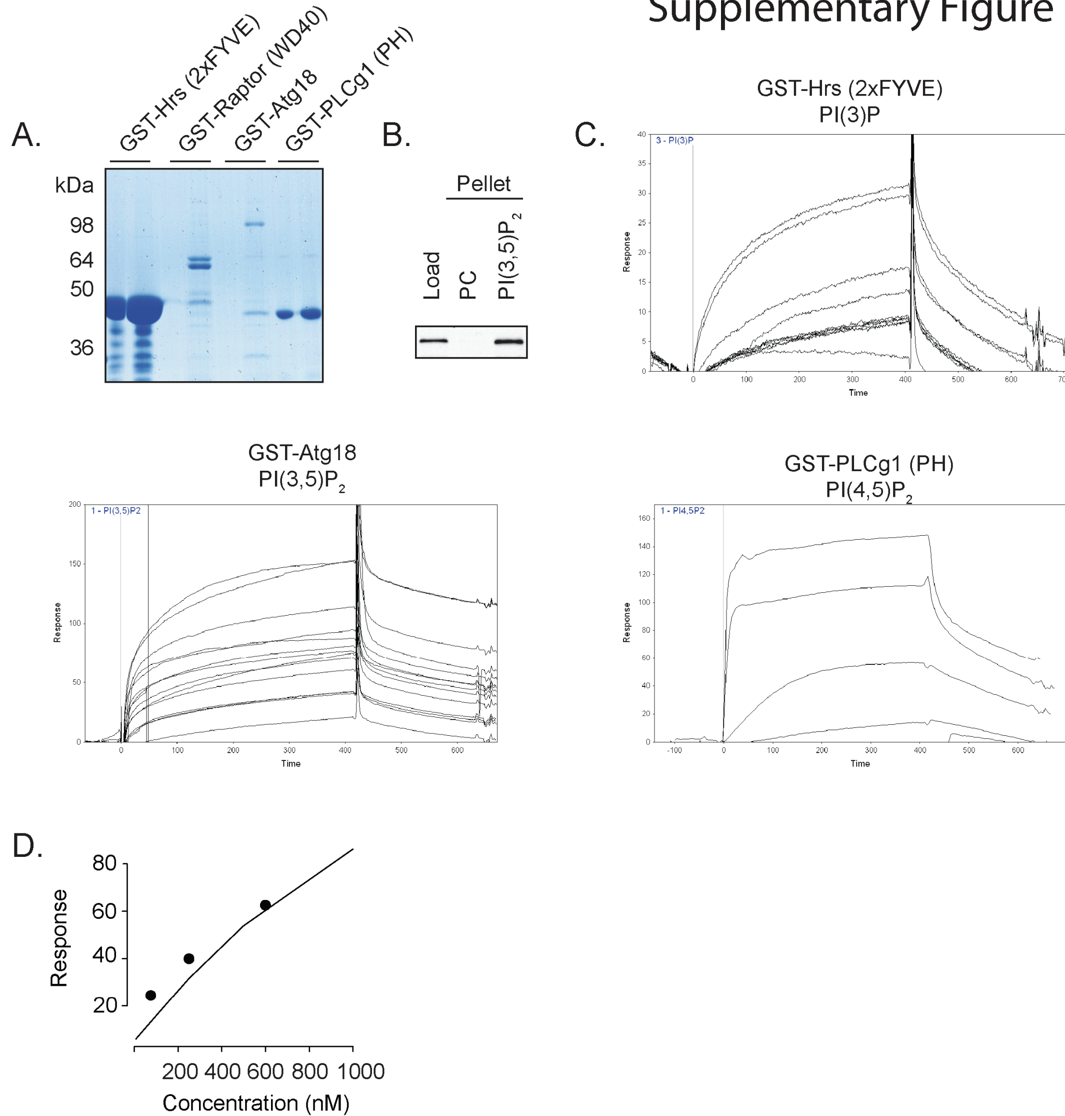
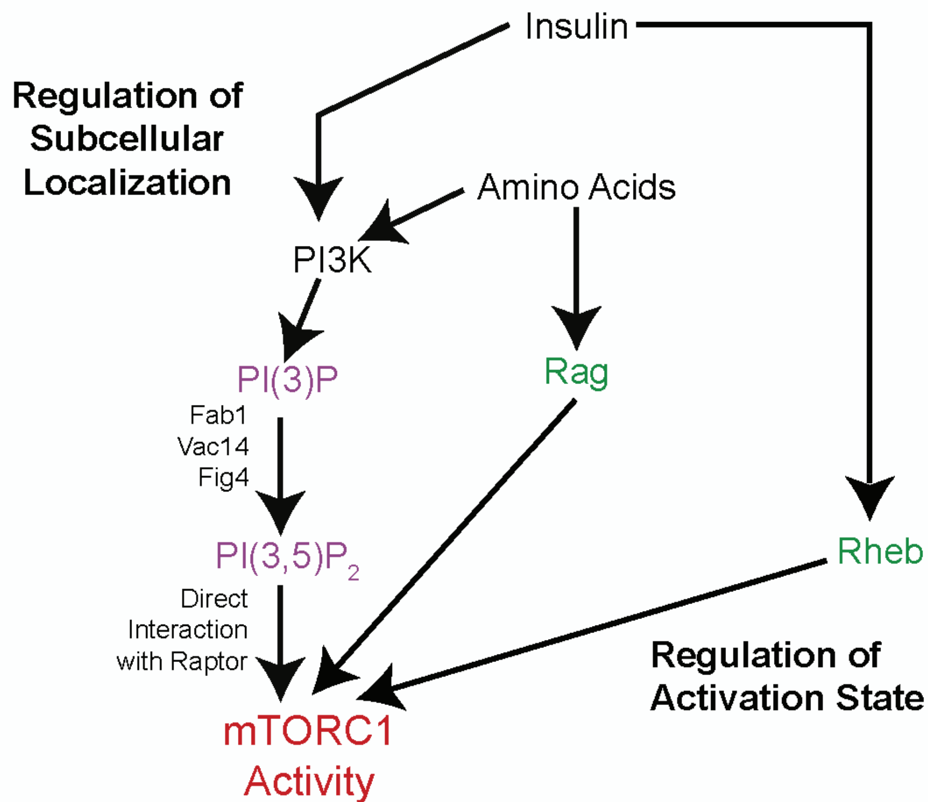


Supplementary Figure 1: GFP-2xFYVE and Lipid Transfection Control Experiments. A) 3T3-L1 Adipocytes were electroporated with GFP-2xFYVE for 24h

and treated with with the indicated concentration of wortmannin or DMSO as described for 1h. Cells were then stimulated with 100 nM Insulin (A) or amino acids (B) for 15 min. C) Knockdown of PI3K-C2α corresponding to experiments presented in Figures 1C and 1F. Knockdown is representative of 3 independent experiments. D and E) For lipid transfection experiments, 3T3-L1 adipocytes were electroporated with GFP-2xFYVE for 24h and treated as described in methods and materials. Three representative cells are shown in D) and quantified in E). Scale bars represent 10 μm. In panels D and E Carrier indicates Carrier 3. F and G) Untransfected serum starved 3T3-L1 adipocytes were treated with 100 nM wortmannin for 1h where indicated and then stimulated with insulin for 15 minutes. F) This panel shows three representative cells. G) Quantification of the data presented in panel F). For all panels, cells were quantified by counting the percentage of cells in which plasma membrane staining of mTOR or GFP-2xFYVE was visible. These data represents three independent experiments in which >200 cells were counted. H) Quantification of effects of knockdown on insulin stimulated pS6K/S6K as described in Figure 3A (n=4). I) Quantification of effects of amino acid stimulation as described in Figure 3D (n=3). For Supplementary Figure 1H-I, only insulin or amino acid stimulated quantification is shown, and the control siRNA was set to 1 for each separate experiment. J) Quantification of lipid transfection effects on pS6K/S6K as described in Figure 4A. Carrier only transfection was set to 1 for each independent experiment (n=3-4). Asterisks for Supplementary Figure 1H-J indicate a one-way t-test compared to either control siRNA (H and I) or carrier only (J) treatments. Asterisks indicate a statistically significant difference ($p<0.05$).



Supplementary Figure 2: Lipid Binding Positive Control Experiments. A) SDS-PAGE gel, stained with coomassie blue showing representative preparations of lipid binding proteins. B) Interaction of GST-Atg18 with PI(3,5)P_2 via liposome precipitation assays. C) Surface Plasmon Resonance traces of positive control interactions with the protein (10nM -2μM protein concentrations), and the interacting lipid indicated. Traces show interactions with varying concentrations of protein. D) Dose response of surface Plasmon resonance experiments described in Figure 5E.



Supplementary Figure 3: Proposed Model for $PI(3,5)P_2$ Regulation of mTORC1.

Note: it is not clear from these studies how Rag/Rheb and the phospholipid changes described in this study may co-operate in the regulation of mTORC1 activity.

Supplementary Table 1: siRNA sequences used in this study. All sequences are stealth modified siRNA duplexes from Invitrogen.

Gene	Oligonucelotide Sequences
Human PIKFYVE (for HEK-293A experiments)	CAGGAAAGGGAATTCTTGAAGAATT GCAGCCCTATCAGATTACCTGAAAT
Mouse PIKFYVE (for 3T3-L1 adipocyte experiments)	GACTGTGAATGAAAGTGGATGTGAA GCCTTCATCAAAGAGTCCTTATTTA
Mouse PI3K-C2α	CAGTAGTTTGCCAACTGGAAGTTCT AAATAGCCTGGATTTGTGCAGTGAT
Mouse VAC14	TCACAATGTCCTTTAAGAGGCGGTC GCTTTGGAAGTGGCATCAATGTCTT
Mouse FIG4	CGCCCTGATTCTTACTGCAGCATTT GCTGAACGTTTGGTGTTCTGACACT
Mouse Vps34	CCTCCACCTGCGAAGGTATTCTAAT TGGCCCTCACCATATGGGATGTGTA