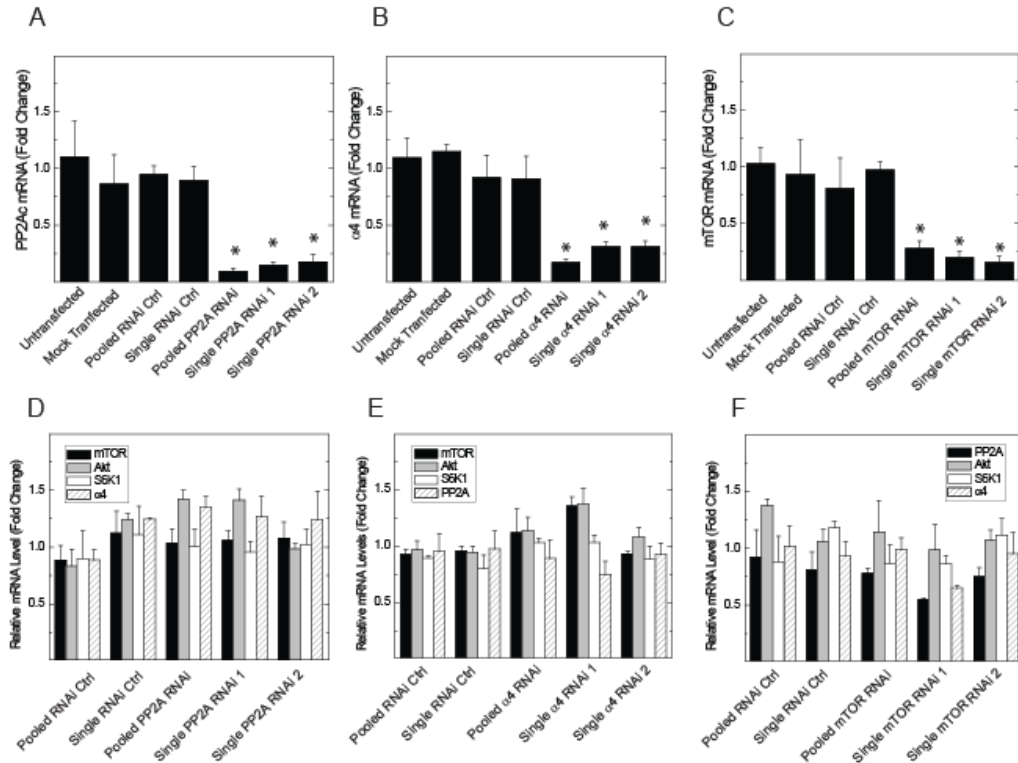


Supplementary Figure 3: Efficacy and Specificity of Pooled and Single siRNA Duplexes:



Supplementary Figure 3: RNA was purified from untransfected A549 cells, or those that were mock-transfected (transfection reagent alone) or siRNA-transfected for 72 h. Preparations of siRNA duplexes included single or pooled duplexes targeting mTOR (30 nM), alpha4 (10 nM), or PP2Ac (10 nM) as indicated in Supplementary Table 1. mRNA levels of the indicated transcripts were detected by real-time PCR. Data are means of fold change in mRNA levels (untransfected controls = 1) from 3 individual experiments ± SEM as determined by the  $\Delta\Delta CT$  method. \*  $p < 0.05$  vs. untransfected control.

Pooled siRNA mixes contain 4 individual siRNA duplexes (Pooled RNAi; Smartpool, Dharmacon). The pooled siRNA control mix contains 4 individual duplexes that do not target mRNAs in the human transcriptome. We obtained two single siRNA duplexes from each pooled siRNA mix, and compared their respective effects on mRNA levels to those of the pooled siRNAs. Panels A-C: Each single or pooled siRNA mixture effectively depletes its target transcript. Panels D-F: None of the single or pooled

siRNAs significantly reduce the levels of other transcripts that encode proteins in the mTOR signaling pathway. Data are means of fold change in mRNA levels (untransfected controls = 1) from 3 individual experiments  $\pm$  SEM. \*  $p < 0.05$  vs. untransfected control.