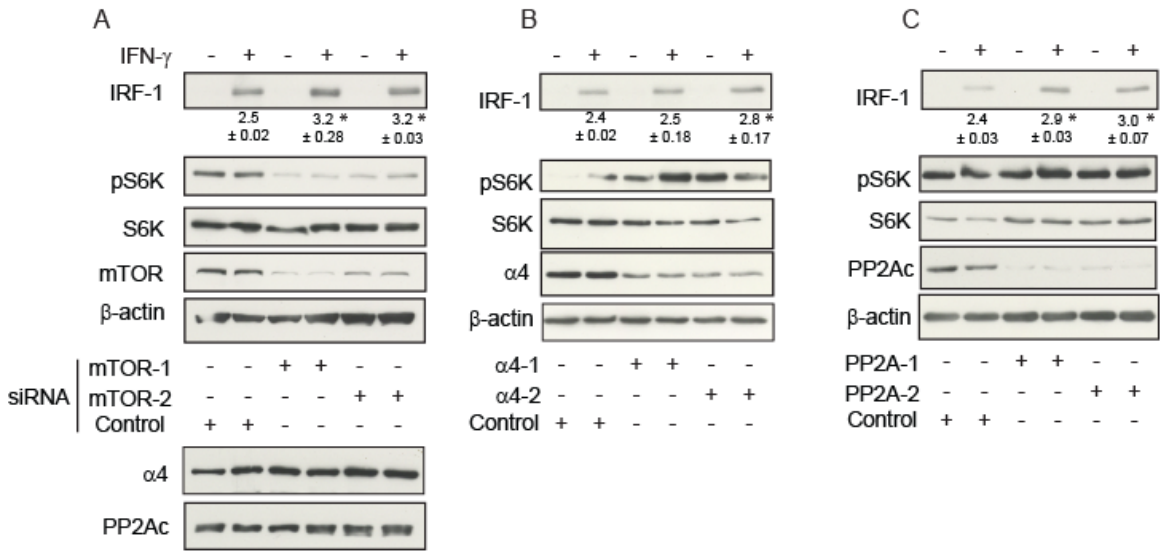
Supplementary Figure 4: Effect of single siRNA duplexes targeting mTOR, α4, or PP2Ac on IRF-1 induction by IFN-γ:



Supplementary Figure 4: A549 cells were transfected with non-targeting single siRNA duplex (Control) or those targeting A. mTOR (30 nM), B. α4 (10 nM), or C. PP2Acα (10 nM) for 72 h before serum withdrawal for 1 h in the absence or presence of rapamycin, incubation without or with IFN-γ for 2 h, and detection of the indicated proteins by Western blot. Sequences of the duplexes are recorded in Table S1. Mean integrated band density for IRF-1 ± SEM (* $p < 0.05$ targeting siRNA vs. non-targeting control, n=3-4 individual experiments) is shown below each Western blot. * $p < 0.05$ *vs.* non-targeting control.

Although siRNA duplex mTOR-1 reduced a4 and PP2Ac levels by 45% (Figure S4), protein levels were unaffected (Figure S5A, bottom panel). Duplex α4-1 was not as effective as α4-2 at reducing IRF-1 induction . As was the case for pooled siRNA (Fig. 5), α4 and PP2Ac depletion with single duplexes enhanced phosphorylation of S6K; depletion of mTOR with single duplexes reduced phosphorylation of S6K.