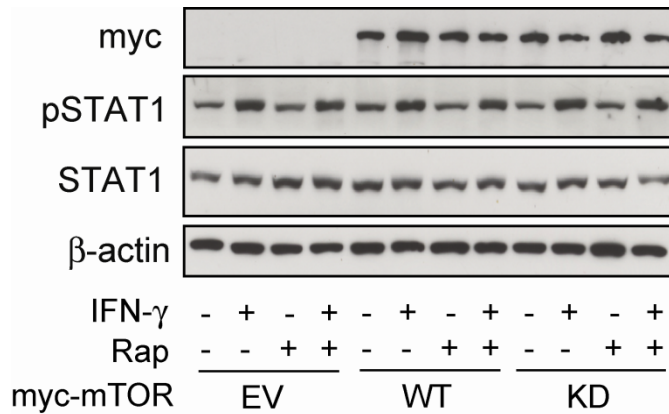
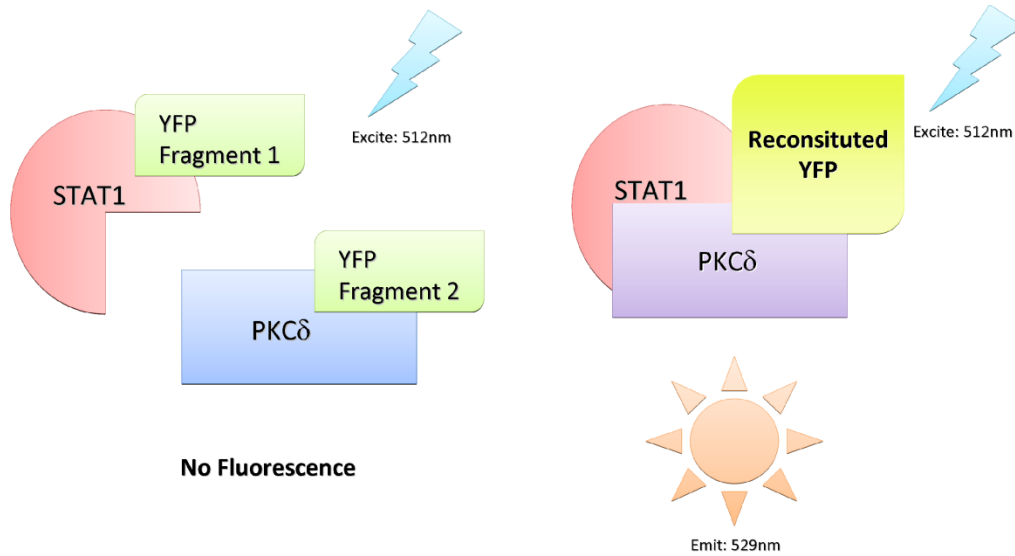


Supplementary Figure 1: Effect of Dominant-negative mTOR on the Phosphorylation of STAT1:



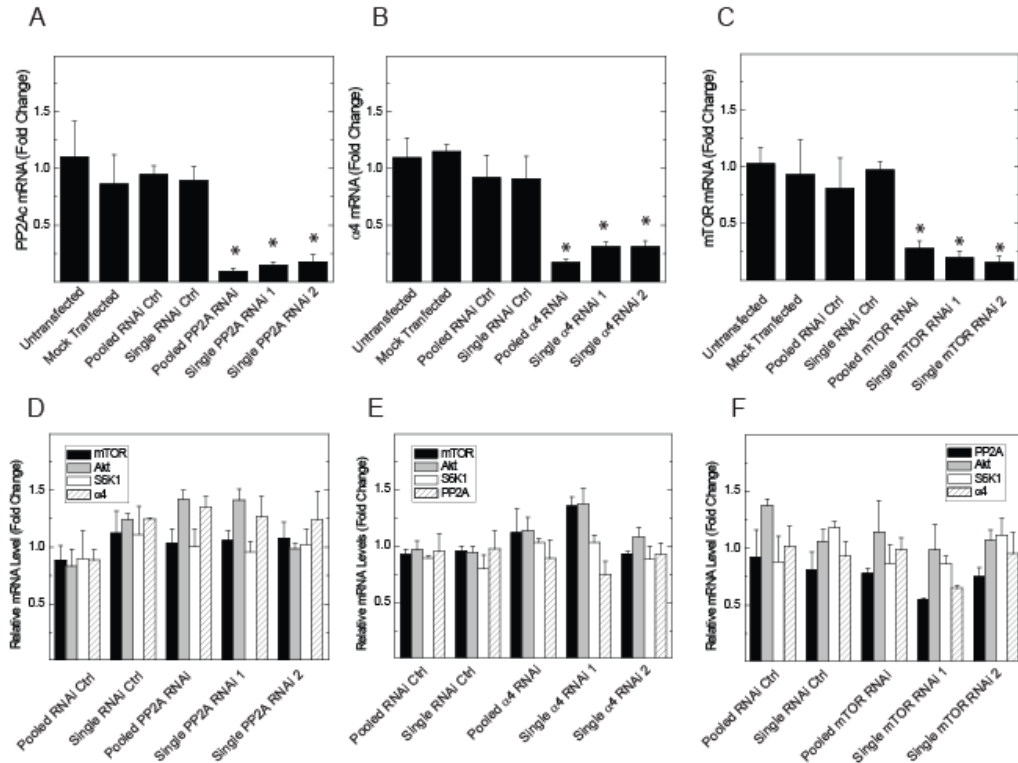
Supplementary Figure 1: HEK 293T cells expressing myc-tagged wild-type (WT) or kinase-dead (KD) mTOR, were serum-deprived for 1 h in the absence or presence of rapamycin before incubation without or with IFN- γ for 30 min. Proteins from whole cell lysates were separated by SDS-PAGE and detected by Western blot analysis. Samples from the same experiment were used for immunoprecipitation of myc-mTOR-associated proteins (Figure 1E). Results are representative of 3 individual experiments.

Supplementary Figure 2: Protein Fragment Complementation Assay:



Supplementary Figure 2: STAT1 and PKC δ , each linked to complementary fragments of yellow fluorescent protein (YFP) were expressed in A549 cells. Physical interaction of the recombinant proteins leads to reconstitution of active YFP permitting detection by fluorescence microscopy.

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

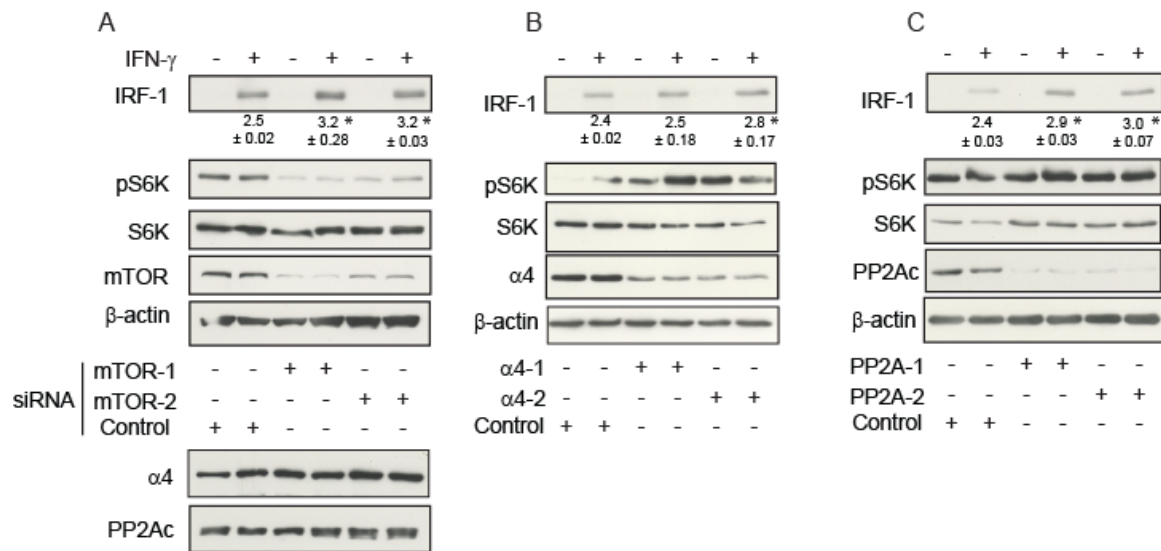


Supplementary Figure 3: RNA was purified from untransfected A549 cells, or those that were mock-transfected (transfection reagent alone) or siRNA-transfected for 72 h. Preparations of siRNA duplexes included single or pooled duplexes targeting mTOR (30 nM), $\alpha 4$ (10 nM), or PP2Ac (10 nM) as indicated 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 \pm 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.

Supplementary Figure 4: Effect of single siRNA duplexes targeting mTOR, $\alpha 4$, 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. $\alpha 4$ (10 nM), or C. PP2Ac (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 \pm 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 $\alpha 4$ and PP2Ac levels by 45% (Figure S4), protein levels were unaffected (Figure S5A, bottom panel). Duplex $\alpha 4$ -1 was not as effective as $\alpha 4$ -2 at reducing IRF-1 induction. As was the case for pooled siRNA (Fig. 5), $\alpha 4$ and PP2Ac depletion with single duplexes enhanced phosphorylation of S6K; depletion of mTOR with single duplexes reduced phosphorylation of S6K.

Supplementary Tables:

Table S1: Dharmacon siRNA targets and source:

siRNA target	Catalogue Number	Entrez Gene ID
Pooled siRNA:		
PP2A α	L-003598-01	5515
α 4	L-011298-00	3476
mTOR	L-003008-00	2475
siControl Non-Targeting siRNA Pool	D-001206-13	N/A
Single siRNA:		
	Catalogue Number	Target Sequence
PP2A α -1	J-003598-9	ccggaauguaguaacgau
PP2A α -2	J-003598-10	acaauaacaccucgugaau
α 4-1	J-011298-06	gcuaucauguggcagaguu
α 4-2	J-011298-07	gggcugaucuucccacaac
mTOR-1	J-003008-11	ggccauagcuagccucaua
mTOR-2	J-003008-12	caaaggacuucgcccauaa
siControl	On-Target plus Non-targeting siRNA #1	ugguuuacaugucgacuaa

Table S2: Antibodies used for Western blot analysis or immunoprecipitations:

Protein	Source
Rabbit anti-STAT1 (Western)	Santa Cruz Biotechnology
Mouse anti-STAT1 α (IP or Western)	Santa Cruz Biotechnology
phospho-STAT1 S727	Upstate
phospho-STAT1 Y701	Upstate
PKC δ	Santa Cruz Biotechnology
Caspase-1	Santa Cruz Biotechnology
IRF-1	Santa Cruz Biotechnology
β -actin	Sigma
GST	Santa Cruz Biotechnology
α 4	Upstate
mTOR	Upstate
PP2Ac	Cell Signaling Technology
4E-BP1	Cell Signaling Technology
mLST8	Cell Signaling Technology
p70 S6 kinase	Cell Signaling Technology
phospho-p70 S6 kinase T389	Cell Signaling Technology
Enolase	Santa Cruz
Acetylated histone H3	Upstate
Rheb	Cell Signaling Technology
Rabbit anti-myc (IP)	Upstate

Mouse anti-myc (Western)	Upstate
Phospho-Akt S473	Cell Signaling
Akt	Cell Signaling

Table S3: Oligonucleotide primers (5'-3') for PCR cloning of STAT1, PKC δ , and PP2A into Gateway entry vector pDONR-221:

Transcript	Forward Primer	Reverse Primer	Template
STAT1-WT	ggggacaagttgtacaaa aaagcaggctgtatgtctc agtggtagcaacttcagcg cagcg	ggggaccactttgtacaa gaaagctgggtcctatact gtgtcatcatactgtcgaa ttct	human lung cDNA
PKC δ	ggggacaagttgtacaaa aaagcaggctatggcgcc gttctcg	ggggaccactttgtacaa gaaagctgggttctaacc ggaacctccatcttc	pcDNA3.1 - PKC δ - WT
PP2A α - WT	ggggacaagttgtacaaa aaagcaggcttcatggacg agaaggtgtcacca	ggggaccactttgtacaa gaaagctgggtcttacag gaagaagtctgggta	pcDNA3 – PP2A α - WT
PP2A α - Y307F	ggggacaagttgtacaaa aaagcaggcttcatggacg agaaggtgtcacca	ggggaccactttgtacaa gaaagctgggtcttacag gaagaagtctgggta	pcDNA3 – PP2A α - Y307F

Table S4: Oligonucleotide primers (5'-3') for Sybr Green-based Real-time PCR:

Transcript	Forward Primer	Reverse Primer
STAT1	tccatcctttggtacaacatgc	cagagagggagcaggtgttt
IRF1	gctgggacatcaacaaggat	gtggaagcatccggtagact
45S rRNA	aacgcctgacacgcacggcacggag	cctgctgttctctcgcgcgtccgag
PP2A α	ggtggcaaatcaccagatac	tctcatgattccctcgaaga
α 4	aattcctccatggcttatcc	cacagcagatttcattgcag
mTOR	gcatccagattgatacctg	tgtctgtgagaagctggtga
Akt	gaagagatggagggtgtcct	atcttcatggcgtagtagcg
S6K	agcacagcaaatcctcagac	tcattgtcacatccatctgc
GAPDH	aagaaggtggtgaagcaggcg	accaggaaatgagcttgaaa

Table S5: Probes for Taqman-based Real-time PCR:

Transcript	Entrez Gene ID	ABI Catalogue Number
hiNOS	4843	HS00167257_m1
hCasp1	834	HS00354836_m1
Fas	355	HS00163653_m1
18S RNA	100008588	4352930E