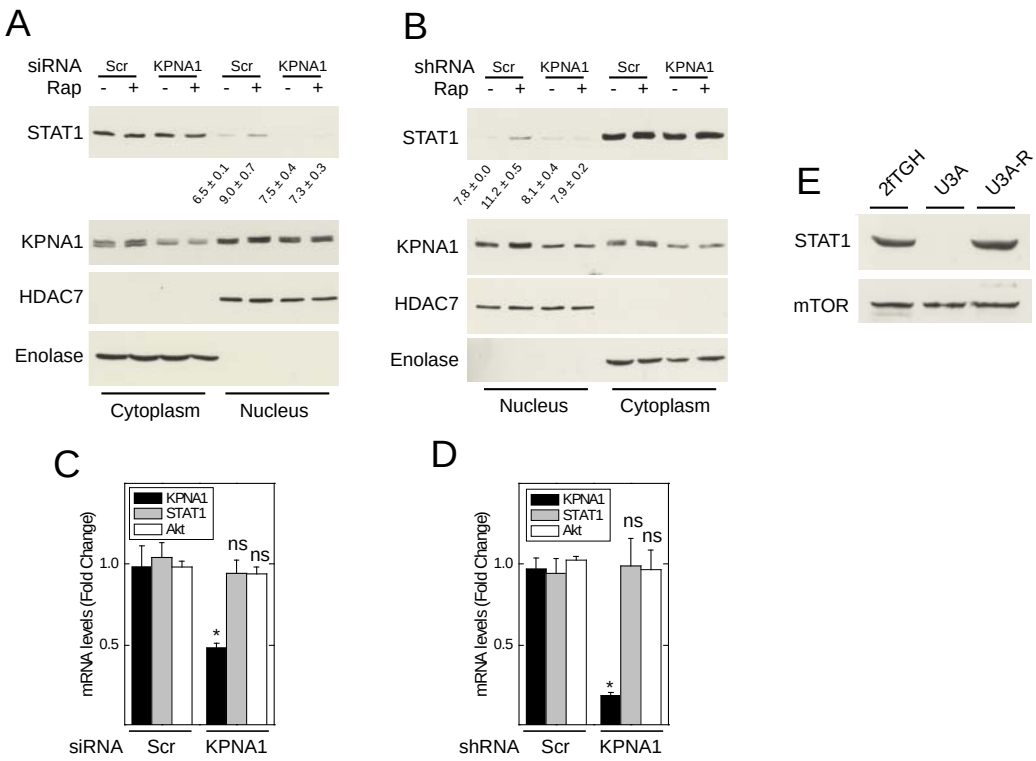
Supplementary Figure 4: KPNA1 is required for rapamyin-induced nuclear import of STAT1 under conditions of constant STAT1 mRNA levels:



Supplementary Figure 4: U3A-R cells, a STAT1-deficient cell line stably reconstituted with STAT1, were transfected with control scrambled (Scr) or KPNA1-targeting (A and C) siRNAs or (B and D) shRNA*. For assessment of nuclear protein levels (A and B), U3A-R cells were incubated for 1 h without or with rapamycin, 50 ng/mL, prior to the generation of nuclear or cytoplasmic fractions (see Experimental Methods), and detection of the indicated proteins by Western blot. Representative gels are shown. Data shown below gels for STAT1 are means of densitometry values for nuclear STAT1 divided by those for histone deacetylase 7 (HDAC7) nuclear loading control (± half the range, n=2). In (C) and (D), cellular KPNA1, STAT1, or Akt mRNA levels were detected by real-time PCR in cultured U3A-R cells transfected with control or KPNA1-targeting (C) siRNA or (D) shRNA. Data are mean fold-change *vs.* control = 1 of triplicate samples (± SEM) as determined by the ΔΔCT method, and are representative of 2 individual experiments. * $p < 0.05$ *vs.* control; ns, not statistically significant. (E) Parental 2FTGH, STAT1-deficient U3A, and STAT1-reconstituted U3A (U3A-R) cells were cultured and homogenized before measurement of STAT1 protein levels by Western blot analysis. mTOR protein levels are shown for comparison.

* STAT1-deficient cells reconstituted with STAT1α (U3A-R) were obtained from Dr. G. Stark (Cleveland Clinic), and propagated as previously described (1). For generation of lentiviral particles, HEK 293T cells were co-transfected with the packaging vectors pMDLg-pREE (1.5 μg), pRSV-Rev (1.5 μg), and pMD2-VSVG (3 μg), along with PLKO.1-puro scrambled (6 μg, Sigma cat #SHC002) or PLKO.1-KPNA1 shRNA (6 μg, Sigma cat #TRCN0000065302, targeting sequence 5'-ccggctgctgaatttcggacaagaactcgagttcttgtccgaaattcagcagtttttg-3'), and 12 mM calcium phosphate in 1 ml HEPES buffered saline (pH 7.05, 50 mM HEPES, 1.5 mM sodium phosphate, 280 mM NaCl). On the following day, the transfection mixtures were replaced with fresh media and viral particles were collected after 24 h. The viral supernatants were then removed, passed through a 0.45 μm filter, and added to U3A-R cells in an equal volume of media containing 4 μg/mL hexadimethrine bromide (Sigma). Cells were incubated with the viral particles for 72 h, before initiation of experimental procedures. For siRNA-mediated knockdown of KPNA1, U3A-R cells were transfected as described in the materials and methods, before initiation of experimental procedures.