Inhibition of translation by IFIT family members is determined by their ability to interact selectively with the 5'-terminal regions of cap0-, cap1- and 5'ppp- mRNAs

Parimal Kumar[1,#], Trevor R. Sweeney[1,#], Maxim A. Skabkin[1,&], Olga V. Skabkina[1,&], Christopher U. T. Hellen[1,*], and Tatyana V. Pestova[1,*]

[1]Department of Cell Biology, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203

[#] These authors contributed equally to this work and are listed in alphabetical order

[&] These authors initiated the project and made equal contributions

***** Corresponding authors: email: tatyana.pestova@downstate.edu

email: christopher.hellen@downstate.edu

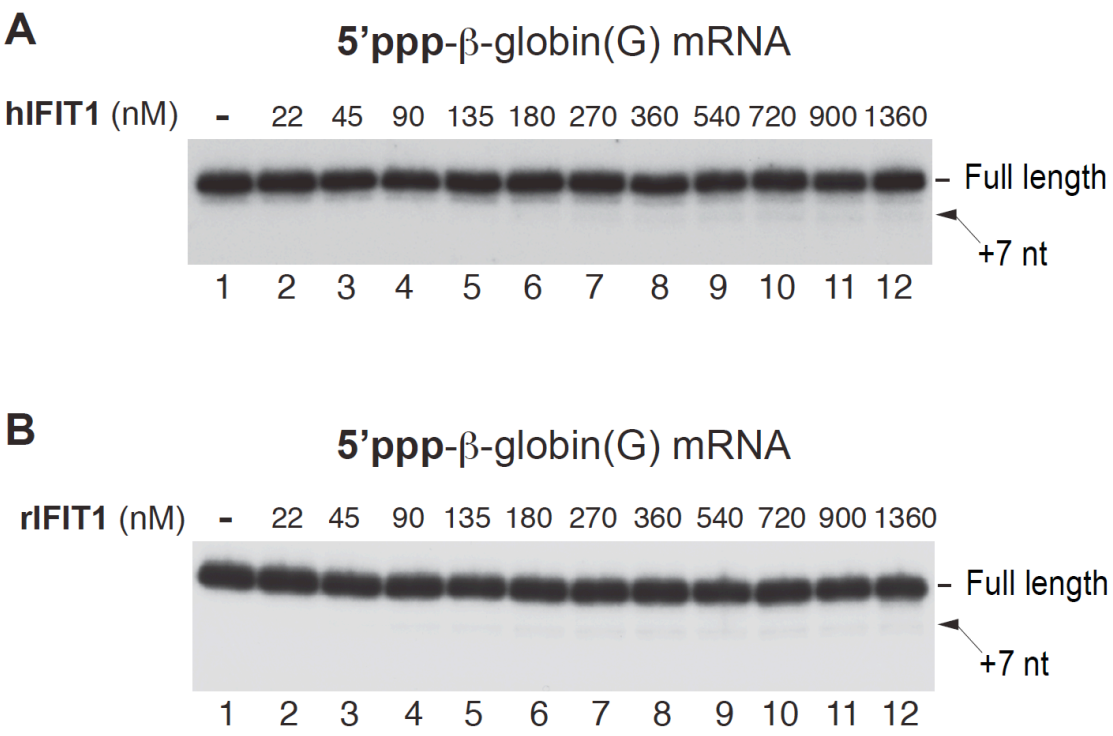

Figure S1. Representative gels of titration of the association of (A) hIFIT1 and (B) rIFIT1 with 5'ppp-β-globin(G) mRNA, assayed by primer extension. Toe-prints induced by the IFIT/mRNA interaction are indicated.

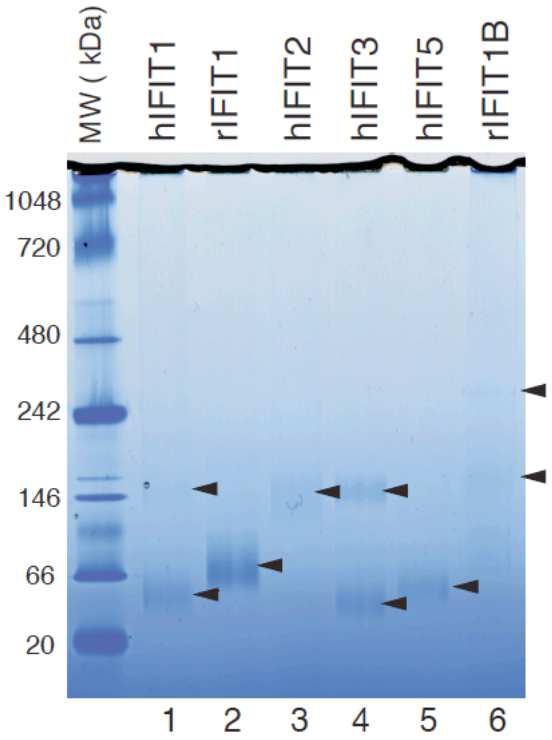

Figure S2. Visualization of purified recombinant IFITs by native gel electrophoresis. The positions of proteins are indicated by arrowheads.

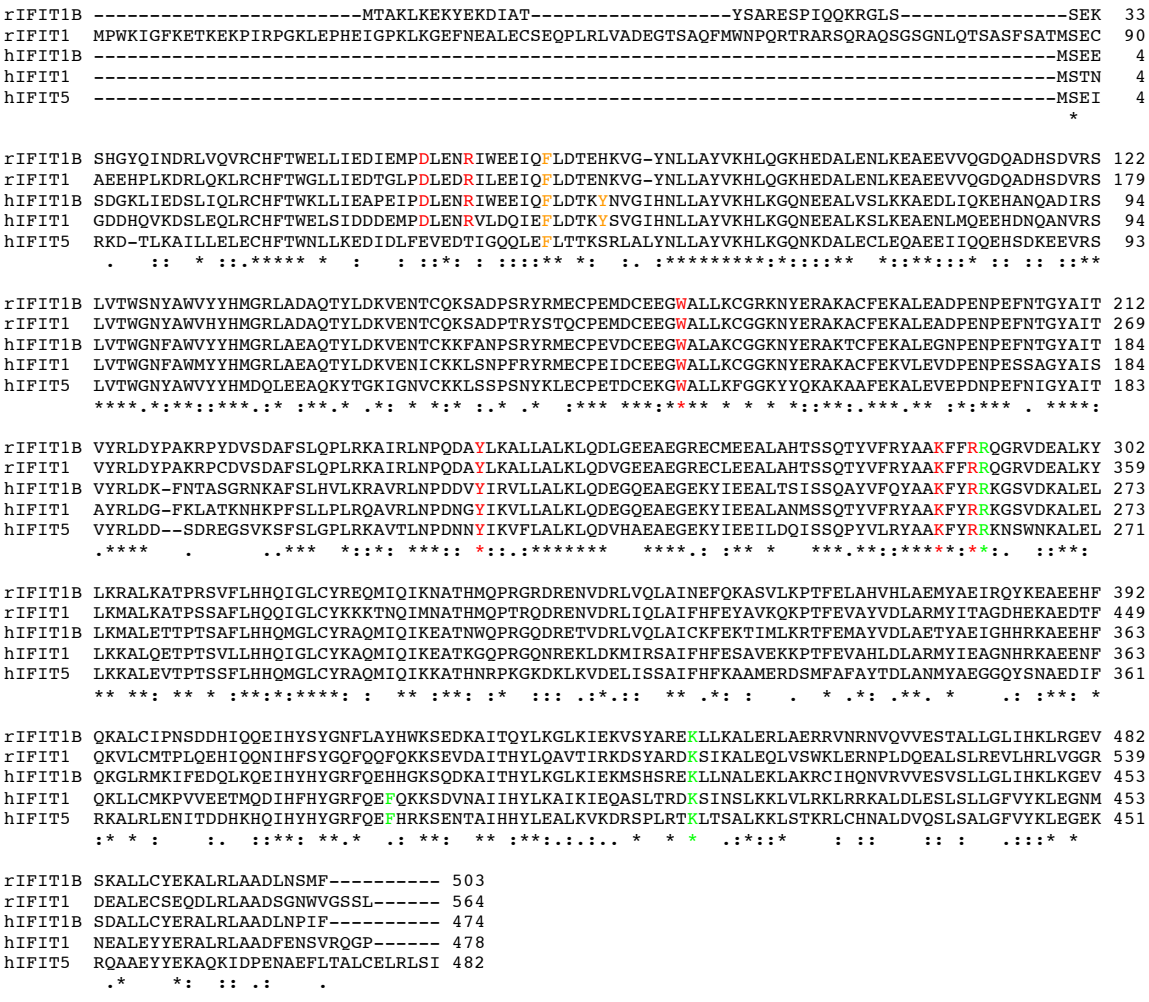

Figure S3. Alignment was performed using ClustalW. Sequences used were rIFIT1B: XP_002718420.1, rIFIT1: XP_002718421.1, hIFIT1B: NP_001010987.1, hIFIT1: NP_001539, and hIFIT5: NP_036552.1. Mutation of residues in red inhibited hIFIT1 binding to cap0-β-globin(G) mRNA. Mutation of residues in green had a weak effect on hIFIT1 binding to cap0-β-globin(G) mRNA. Single mutation of residues in orange decreased the intensity of the reverse transcriptase stop induced by hIFIT1 binding to cap0-β-globin(G) mRNA but did not block the protein's ability to inhibit 48S initiation complex assembly. Double mutation of residues in orange inhibited binding of hIFIT1 to cap0-β-globin(G) mRNA.

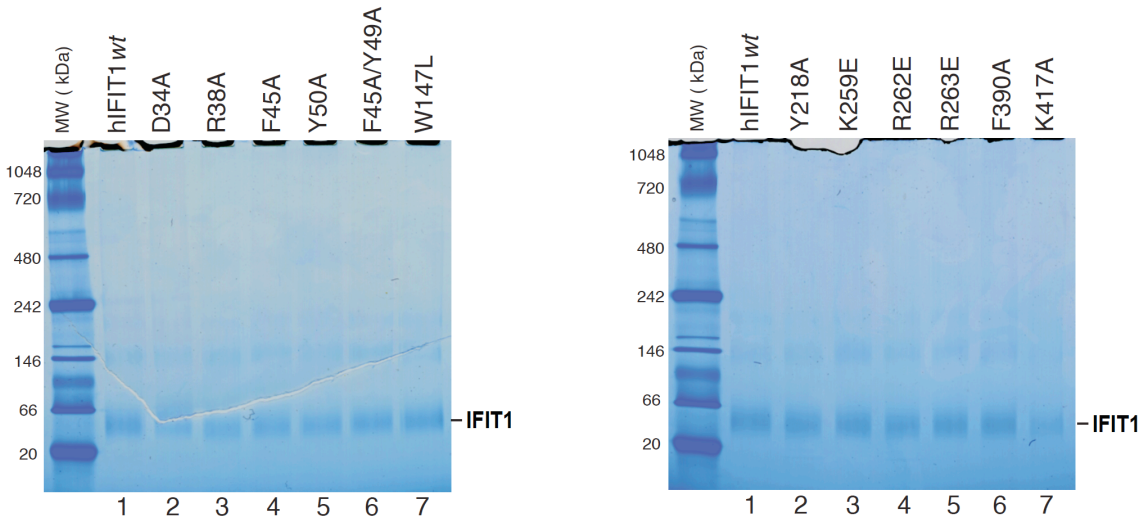

Figure S4. Visualization of purified recombinant IFIT1 mutants by native gel electrophoresis.

Table S1. Identification of IFIT1B by LC/nanospray tandem mass-spectrometry of tryptic peptides

Deduced sequence	Amino acid residues
SHGYQINDR	44 - 52
IWEEIQFLDTEHK	68 - 80
VGYNLLAYVK	81 - 90
HLQGKHEDALENLK	91 - 104
HEDALENLKEAEEVVQGDQADHSDVR	96 - 121
EAEEVVQGDQADHSDVRSLVTWSNYAWVYYHMGR	105 - 138
SLVTWSNYAWVYYHMGRLADAQTYLDK	122 - 148
LADAQTYLDKVENTCQK	139 - 155
ALEADPENPEFNTGYAITVYRLDYPAK	195 - 221
RPYDVSDAFSLQPLRK	222 - 237
LNPQDAYLKALLALK	241 - 255
ALLALKLQDLGEEAEGR	250 - 266
ECMEEALAHTSSQTYVFR	267 - 291
QGRVDEALK	293 - 301
SVFLHHQIGLCYR	313 - 325
EQMIQIK	326 - 332
NATHMQPR	333 - 340
LVQLAINEFQK	350 - 360
ASVLKPTFELAHVHLAEMYAEIRQYKEAEEHFQK	361 - 394
SEDKAITQYLK	423 - 433
NVQVVESTALLGLIHKLR	462 - 479
LAADLNSMF	495 - 503

* Amino acid residues of unique peptides are numbered according to the sequence of *O. cuniculus* IFIT1b (NCBI Reference sequence XP_002718420.1).

There are two rabbit IFIT1-like proteins (NCBI Reference sequences XP_002718421.1 and XP_002718420.1) and we have designated them rIFIT1 and rIFIT1B, respectively, because of shared gene synteny with human IFIT1 and IFIT1B, and because of the greater sequence identities of rIFIT1B with hIFIT1B (68.9%) than with hIFIT1(58.5%) and of rIFIT1 with hIFIT1 (61.6%) than with hIFIT1B (59.2%), as determined by CLUSTAL-W2 alignment.