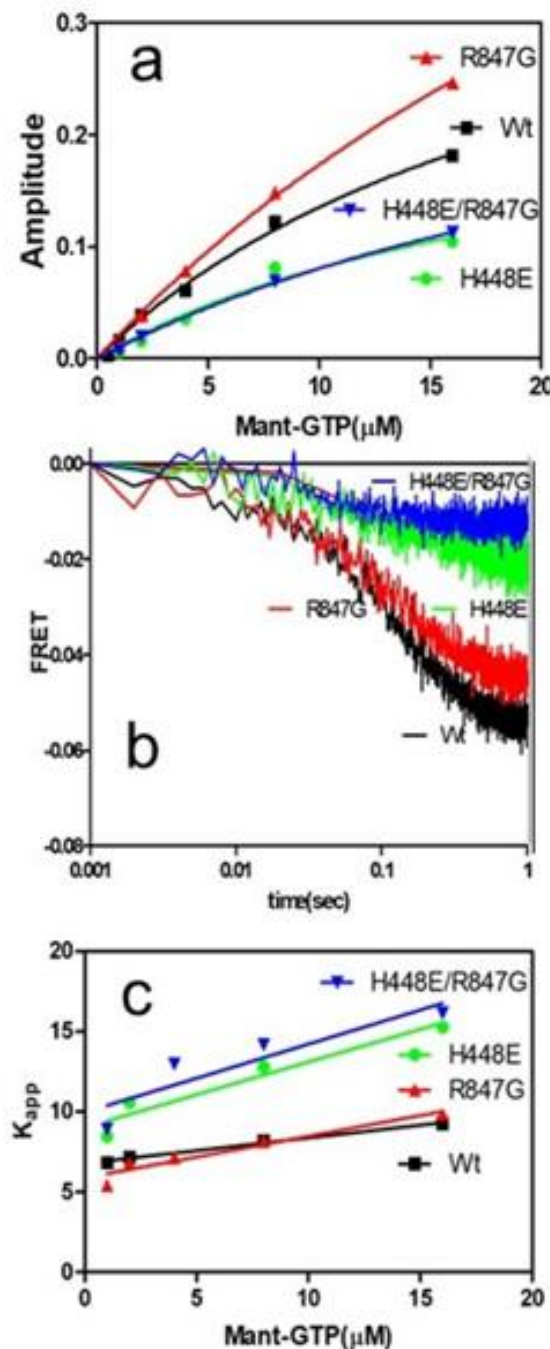


**Figure S1** 3D structure of *Thermus thermophilus* IF2 bound to GDP (blue). The IF2 domains are color-coded: G2 (red), G3 (yellow) and C1 (green). The position, of the His residue corresponding to *E. coli* His448, located at the tip of switch II, is indicated by the red arrow. The figure is from pdb file 4KJZ.



**Figure S2** Interaction of Mant-GTP with IF2 wt (black), IF2 H448E (green), IF2 R847G (red) and IF2 H448E/R847G (blue) mutants. **a)** fluorescence amplitude changes upon binding Mant-GTP to IF2 molecules as a function of increasing concentrations of the nucleotide triphosphate; **b)** stopped-flow kinetic analysis of Mant-GTP dissociation from IF2 molecules monitored by the decrease of the FRET fluorescence emission using as observable the FRET signal generated by exciting the intrinsic Trp residue of IF2; **c)** variation of the  $K_{app}$  of Mant-GTP binding to IF2 molecules as a function of increasing concentrations of the nucleotides. The experiments were carried out with a Kintek Model SF-2004 stop-flow apparatus (Figure S4). The IF2 molecules bearing the H448E substitution bind GTP faster and dissociate GTP more slowly than IF2 wt and IF2 R847G. Thus, the reduced fluorescence amplitude observed with these mutants suggests that the conformation in these mutants is somewhat altered.

5'GGGAAUUCAAAAUUUAAAAGUUAAC**AGGU**AUACAUACU**AUG**UUUACGAU  
UACUACGAUCUUCUUCACU**UAA**CGCGUCUGCAGGCAUGCAAGCU-3'.

5' - gggaauucaaaaaaaguuuacacggaauuacauacu AUG M P T I

ACU ACG AUC UUC UUU ACG AUC UUC UUU ACG AUU ACU ACG AUC UUC  
T T I P T T I P T T I T T I P  
UUU ACG AUU ACG AUC UUC UUU ACG AUU ACU ACG AUC UUC UUU  
P T I T T I P T T I T T I P P  
ACG GGG CUG GUU CCG CGU GGA UCC UCU AGA GUC GAC CUG CAG GAG  
T G L V P P R G S S R V D L Q E  
CAG CGG AGC GUG AAA ACG CGC GUC AGC CUC GAC GAU UUG UUC GAA  
Q R S V K T R V S L D D L F E  
CAA AUC AAG CAA GGC GAA AUG AAA GAG CUG AAC UUG AUC AUA  
Q I K Q G E M K E L N L I V K  
GCC GAU GUU CAA GGA UCG GUU GAG CGG CUG GUU GCC GCC UUG CAA  
A D V Q G S V E A L V A A L Q  
AAA AUC GAU GUC GAA GGC GUG CGG GUG AAA AUC AUC CAU GCG GCG  
K I D V E G V R V K I I H A A  
GUC GGC GCC AUC ACC GAA UCG GAU AUU UCA CUG GCG ACG GCA UCG  
V G A I T E S D I S L A T A S  
AAC GCU AUC GUC AUU GGC UUU AAC GUC CGC CGG GAU GCG AAU GCG  
N A I V I G F N V R P D A N A  
AAG CGC GCC GCU GAA UCG GAA AAA GUC AUC AUC CGC CUC CAU CGC  
K R A A E S E K V D I R L H R  
AUC AUU UAC AAC GUC AUU GAG GAA AUU GAA GCG CGC AUG AAA GGG  
I I Y N V I E E I E A A M K G  
AUG CUC GAC CGG GAA UAC GAA GAG AAA GUC AUC GGC CAA GCG GAA  
M L D P E Y E E K V I G Q A E  
GUG CGG CAA ACG UUC AAA GUG UCC AAA GUU GGC ACG AUC GCC GGC  
V R Q T F K V S K V G T I A G  
UGC UAU GUC ACC GAC GGC AAA AUC ACC CGC GAC AGC AAA GUG CGC  
C Y V T D G K I T R D S K V R  
CUC AUC CGC CAA GGC AUC GUC GUG UAC GAA GGC GAA AUC GAU UCG  
L I R Q G I V V Y E G E I D S  
CUC AAA CGG UAU AAA GAG GAU GUG CGC CAA GUG GCG CAA GGA UAU  
L K R Y K E D V R Q V A Q G Y  
GAG UGC GGC UUG ACG AUC AAA AAC UUC AAU GAC AUU AAA GAA GGG  
E C G L T I K N F N D I K E G  
GAC GUU AUU GAA GCG UAC GUC AUG CAG GAA GUG GCU CGG GCA UGA  
D V I E A Y V M Q E V A R A

gggcuuugccgatgcaagcu (A) 3' gcuu - 3'

a) Sequence of 022mRNA:

**b)** Sequence of 027IF2CmRNA. The Shine-Dalgarno sequence, the initiation and termination triplets are written in bold and underlined. The 5'UTR and 3'UTR are in lower case letters and the coding bases in capital letters. The amino acids of the 027 peptide (bold letters) and *B. stearothermophilus* IF2C domain (non-bold) sequences are indicated below the corresponding codons. The underlined nucleotide sequence corresponds to the spacer encoding a thrombin-sensitive peptide. From [2]



**Fig. S4** Left: Biologique SFM-400 quench-flow apparatus used to analyze the kinetics of IF2-dependent GTP hydrolysis and initiation dipeptide formation in the presence of IF2 wt and IF2 mutants. Right: Kintek Model SF-2004 stop-flow apparatus used for studying the interaction of IF2 wt and IF2 mutants with Mant-GTP.

## References

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