SUPPLEMENTARY DATA

Eukaryotic translation initiation factor eIF5 promotes the accuracy of start codon recognition by regulating P_i release and conformational transitions of the preinitiation complex

Adesh K. Saini[1,3,†*], Jagpreet S. Nanda[2*], Pilar Martin-Marcos[1], Jinsheng Dong[1], Fan Zhang[1], Monika Bhardwaj[3], Jon R. Lorsch [2,†], and Alan G. Hinnebusch [1,†]

[1]Laboratory of Gene Regulation and Development, Eunice K. Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA. [2]Laboratory on the Mechanism and Regulation of Protein Synthesis, Eunice K. Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA. [3]Shoolini University of Biotechnology and Management Sciences, Department of Biotechnology, Bajhol, Solan, India.

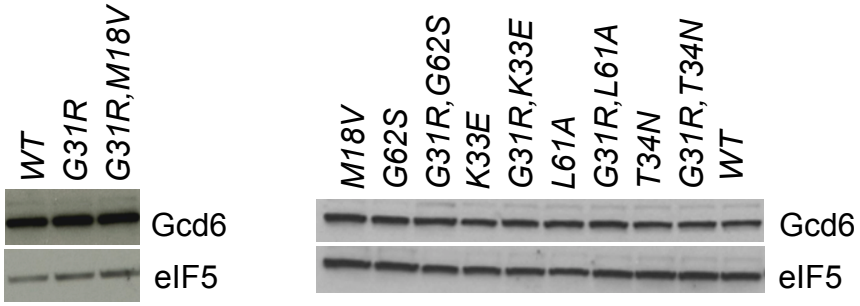

Figure S1. *TIF5* Ssu$^-$ substitutions do not alter steady-state levels of eIF5 in vivo. Derivatives of *his4-301 tif5Δ* strain ASY100 containing the indicated *TIF5* alleles on sc *LEU2* plasmids except *T34N*, which was expressed from a hc *LEU2* plasmid to match the expression level of FLAG-tagged WT eIF5 and containing (*left panel*) or lacking (*right panel*) *TIF5*$^+$ on a *URA3* plasmid were cultured in synthetic complete medium lacking leucine and uracil (SC-LU) or SC-L, respectively. WCEs were prepared under denaturing conditions, resolved by SDS-PAGE and subjected to Western blot analysis using antibodies against FLAG (eIF5) and Gcd6.

Supplementary Figure 2_Saini et al

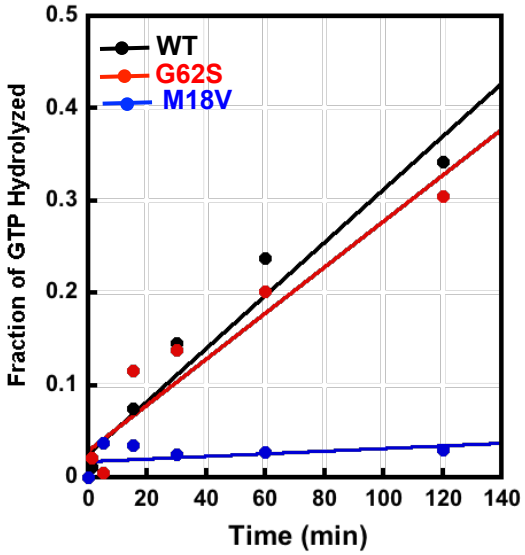

Figure S2. Effects of eIF5 Ssu$^-$ mutants G62S and M18V on GTP hydrolysis by isolated eIF2•GTP•Met-tRNA$_i$ ternary complexes. TC was assembled with [γ-^{32}P]-GTP and GTP hydrolysis was initiated by addition of a saturating concentration of WT, G62S or M18V forms of eIF5. Reactions were stopped at different times with EDTA. The amount of P_i formed over time was analyzed as described in Material and Methods. Rates of GTP hydrolysis are shown for TC with WT eIF5 (black circles; 0.0028 min^{-1}), G62S eIF5 (red circles; 0.0024 min^{-1}), and M18V eIF5 (blue circles; 0.00013 min^{-1}).