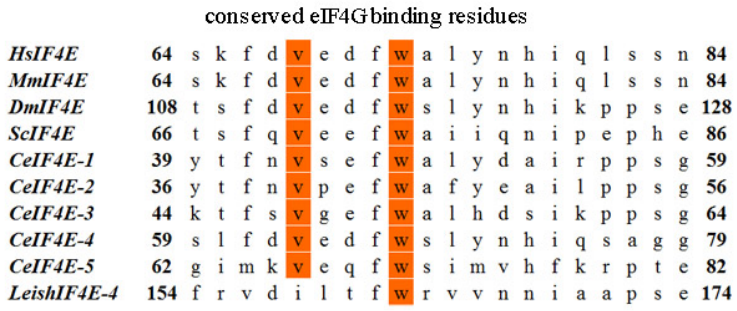
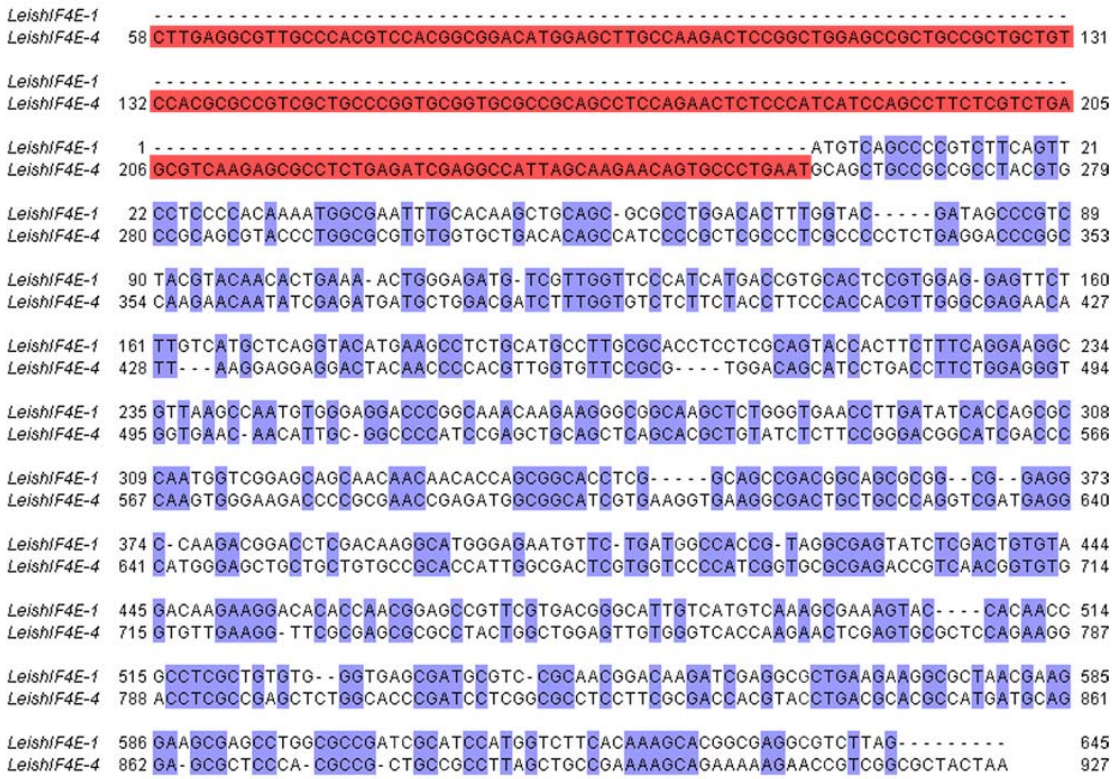
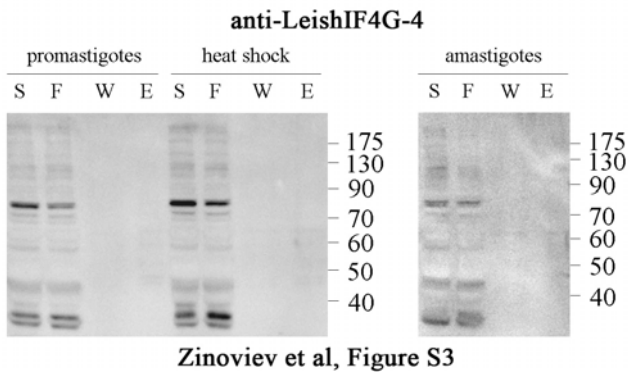


Supplemental Figure S1. **Trp73 (position in the human eIF4E) is required for the interaction with eIF4G, is conserved in LeishIF4E-4.** The multiple sequence alignment was performed in ClustalW, using eIF4E orthologues fromn different species (*H. sapiens, M. musculus, D. melanogaster, S. cerevisiae, C. elegans, L. major*).

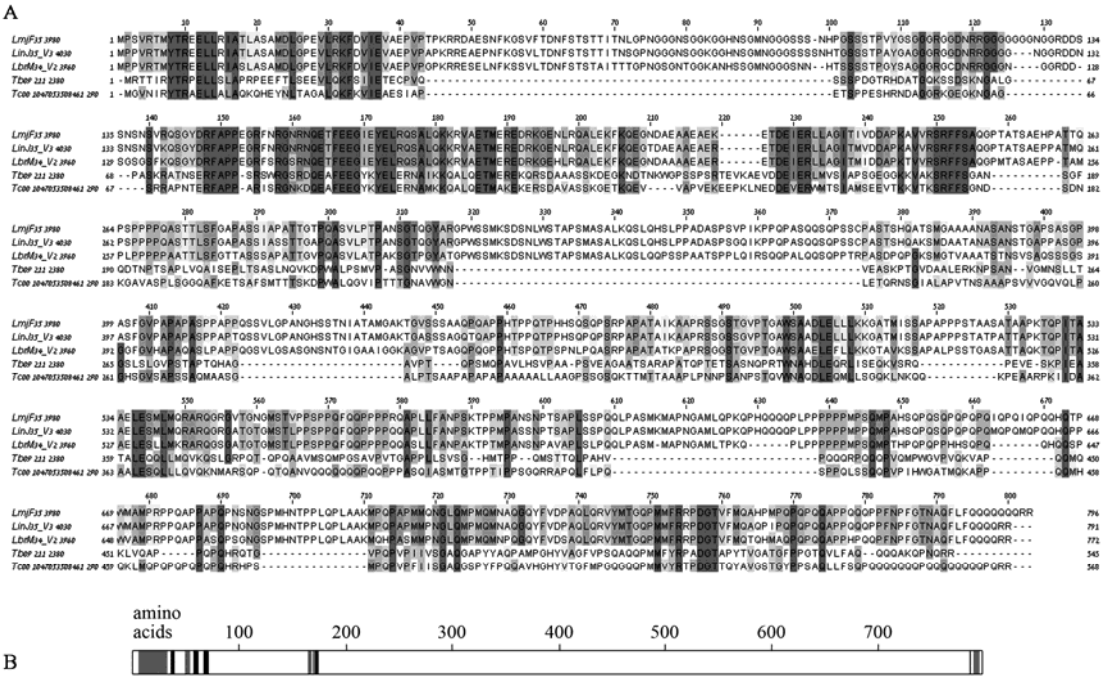


Zinoviev et al, Figure S2

Supplemental Figure S2. The N-terminal part (1-258nt) of LeishIF4E-4 has no equivalent in LeishIF4E-1. The multiple sequence alignment was performed in ClustalW, using the nucleotide sequence for LeishIF4E-1 and LeishIF4E-4. Red – the non homologous part, corresponding to the first 86aa, blue – homologous parts.

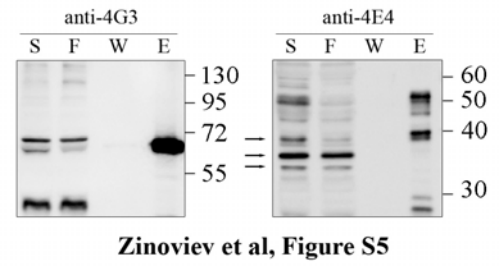


Supplemental Figure S3. LeishIF4E-1 does not interact with LeishIF4G-4. (A) The SBP-tagged LeishIF4E-1 complex was affinity purified from transgenic *L. amazonensis* promastigotes grown at normal conditions or after a 2h heat shock treatment, and from axenic-amastigotes. Supernatants of cell extracts (S) were loaded on streptavidin-Sepharose beads and the unbound proteins were removed (F). The beads were washed (W) and eluted (E). The lanes contain 1% (S, F) or 20% (W, E) of the total respectable fraction proteins. All the proteins were resolved on SDS-PAGE (10-15%) and subjected to western blot analysis using specific antibodies against LeishIF4G-4. Proteins from the same experiments were subjected to analysis with anti-LeishIF4E-1 and the results were similar to those shown in Fig. 2A, 3C and 5A, C, D.

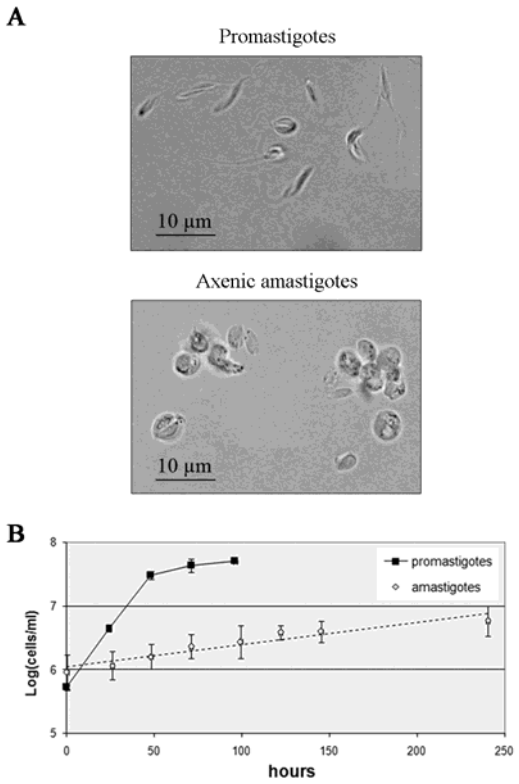


Zinoviev et al, Figure S4

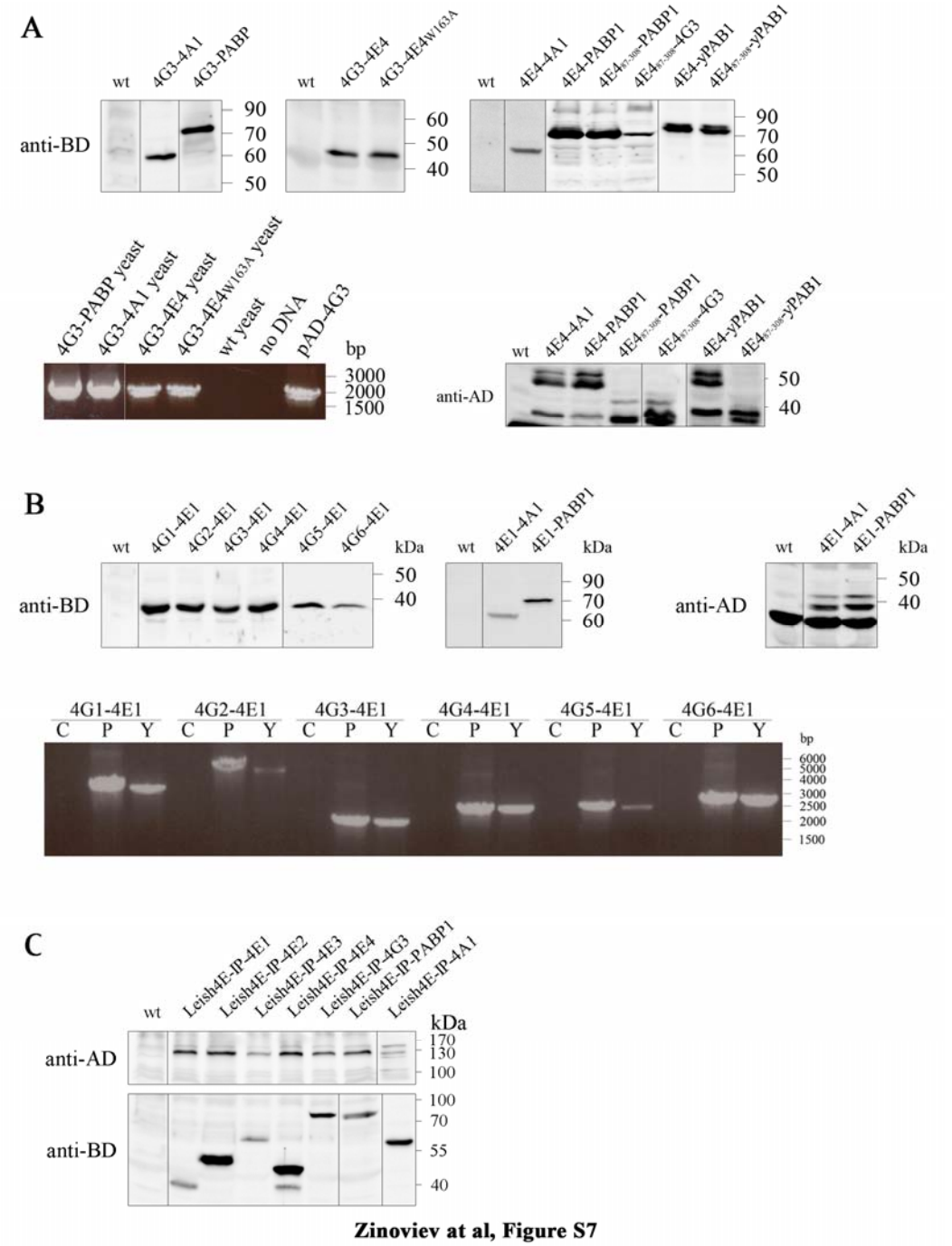
Supplemental Figure S4. A novel 4E-interacting protein in *Leishmania*. (A) Orthologues of the novel 4E-IP from different *Leishmania* and *Trypanosoma* strains were aligned using ClustalW (*L. major* - LmjF35.3980, *L. braziliensis* - LbrM34_V2.3960, *L. infantum* - LinJ35_V3. 4030, *T. brucei* - Tb09.211.2380, *T. cruzi* - Tc00.1047053508461.290). **(B)** A schematic structure prediction of the *L. major* 4E-IP that was produced by the PSI-PRED server. Grey and black boxes mark alpha helixes and beta sheets, respectively. White areas represent the unstructured parts of the protein.



Supplemental Figure S5. LeishIF4G-3 pulls down the largest LeishIF4E-4 band. *L. amazonensis* transgenic promastigotes expressing FLAG tagged LeishIF4G-3 were sonicated and the soluble supernatant (S) was loaded on FLAG-agarose beads. After incubation the unbound proteins were removed (F) and the beads were washed (W) and eluted (E). The lanes contain 1% (S, F) or 20% (W, E) of the total respectable fraction proteins. The proteins were resolved on SDS-PAGE (10-15%) and subjected to western blot analysis using specific antibodies against LeishIF4E-4 and LeishIF4G-3. Arrows indicate the three specific LeishIF4E-4 bands.



Supplemental Figure S6. Transformation of *Leishmania amazonensis* promastigotes into axenic-amastigotes. (A) Promastigotes and axenic-amastigotes, nine days after differentiation was induced, were viewed by phase microscopy. **(B)** Growth curves of promastigotes and axenic-amastigote cells were counted daily. Each point in the graph is an average of at least three counts, the standard deviation is indicated. The axenic amastigote curve represents differentiated cells (after 5 days at 33^{o}C and pH 5.5).



Supplemental Figure S7. Expression of proteins that were tested by the yeast two-hybrid assays. Proteins were extracted from all yeast strains, resolved on SDS-PAGE (12%) and subjected to western analysis by specific antibodies against the BD and the AD (Santa Cruz). For some of the genes the presence of the ORFs was detected by PCR with specific primers (Table S2). The analysis corresponds to yeast strains presented in **(A)** Fig. 1B, **(B)** Fig. 2B and **(C)** Fig. 4A.

Supplementary Materials and Methods

Construction of plasmids for pull-down analysis

The coding regions of LeishIF4E-1 and LeishIF4E-4 were amplified and cloned into pSNSAP1 (Aphasizhev et al. 2003), generating pSNSAP1-LeishIF4E-1 or LeishIF4E-4, in which the coding regions of the target proteins were cloned in frame to the tags. The tag was shortened by introducing a stop codon and an SpeI restriction site into pSNSAP1-LeishIF4E-1 following the Streptavidin Binding Peptide (SBP), generating pSBP-LeishIF4E-1-SpeI. To fuse SBP-LeishIF4E-1 with intergenic regions derived from the Hsp83 genomic cluster that assure expression in amastigotes, the SBP-LeishIF4E-1 was excised by cleavage with BamHI and SpeI, and further ligated into the BamHI and XbaI sites of the pX-H-CAT-H expression cassette, which provides the Hsp83 RNA processing signals in the pX transfection vector of *Leishmania* (David et al. 2010). The resulting plasmid was pX-H-LeishIF4E1-SBP-H. The coding region of LeishIF4E-4 and Leish4E-IP were cloned into the new tagging vector as well, generating pX-H-LeishIF4E-4-SBP-H and pX-H-Leish4E-IP-SBP-H.

The coding region of LeishIF4G-3 was amplified by PCR with primers that included a FLAG tag sequence at their 3' end followed by a stop codon and BamHI (5') and XbaI (3') sites. The PCR product was cloned into the expression cassette pX-H-CAT-H as described above, yielding pX-H-LeishIF4G3-FLAG-H plasmid.

Expression of fusion proteins in yeast

Expression of the proteins tested in the yeast two-hybrid assay was monitored by western blot analysis. Log phase yeast cultures (O.D.600=10) were harvested, washed with 20% TCA and resuspended in 200 µl 20% TCA. Glass beads were then added and the cells were vortexed for 5min. The glass beads were discarded and the proteins were centrifuged at 13,000g for 10min. The pellet was resuspended in SDS-PAGE sample buffer, boiled for 2min and centrifuged for additional 2min at 13,000g. The resulting supernatants (OD600=1.0) were separated by SDS–PAGE (10-15%) and the gels were blotted and exposed to specific antibodies against the AD and BD domains (Santa Cruz).

FLAG pull down *in vivo*

For pull-down assays using the FLAG tag, cells expressing LeishIF4G-3-FLAG (0.6-1x10^9) were harvested, washed, resuspended in FLAG Binding Buffer (FBB) (50mM Tris pH 7.4, 150mM NaCl, 1mM EDTA) and lysed by sonication. Supernatants were agitated with anti-FLAG-M2-Agarose beads (Sigma) for 2 h at 4^{o}C. The beads were washed with FBB containing 0.1% triton and the proteins were eluted by boiling in sample buffer lacking β-mercaptoethanol.

References

Aphasizhev, R., Aphasizheva, I., Nelson, R.E., Gao, G., Simpson, A.M., Kang, X., Falick, A.M., Sbicego, S., and Simpson, L. 2003. Isolation of a U-insertion/deletion editing complex from Leishmania tarentolae mitochondria. EMBO J 22(4): 913-924.

David, M., Gabdank, I., Ben-David, M., Zilka, A., Orr, I., Barash, D., and Shapira, M. 2010. Preferential translation of Hsp83 in *Leishmania* requires a thermosensitive polypyrimidine-rich element in the 3' UTR and involves scanning of the 5' UTR. RNA 16(2): 364-374.