SUPPLEMENTARY DATA

MATERIAL AND METHODS

Translation Assay

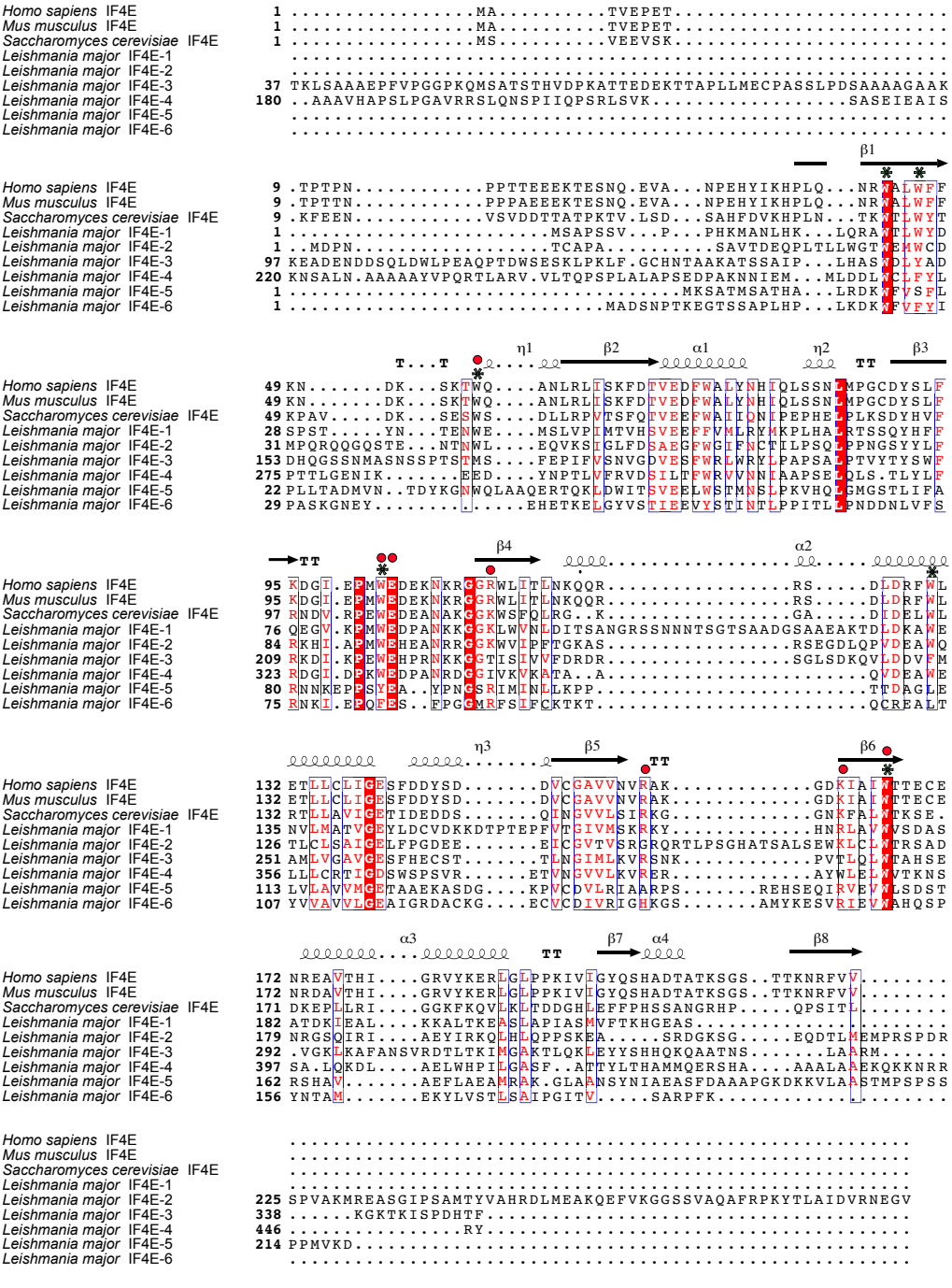
We performed translational assays as previously described in (1), with the following modifications. We cultured HEK293T cells in Dulbecco's Modified Eagle's Medium (DMEM, Lonza), supplemented with 10% FBS (Thermo Scientific) and penicillin/streptomycin (Lonza) to 70-80% confluence in 6-well plates. We transfected 500 ng of a bicistronic reporter construct pFL-EMCV-IRES-RL containing the firefly luciferase followed by the EMCV IRES and the renilla luciferase. Along the dual luciferase reporter, we co-transfected 500 ng of the pcDNA3-eGFP plasmid, or the plasmids encoding 4E-IP1 fragments (pcDNA3-Leish4E-IP1-eGFP) or mouse 4E-BP1 fragment (pcDNA3-4E-BP-eGFP) using polyethyleneimine (PEI). Twenty-four hours post-transfection, we lysed and measured the luciferase activity with a dual luciferase reporter assay system (Promega) using an EnVision 1 plate reader.

Yeast two-hybrid assay

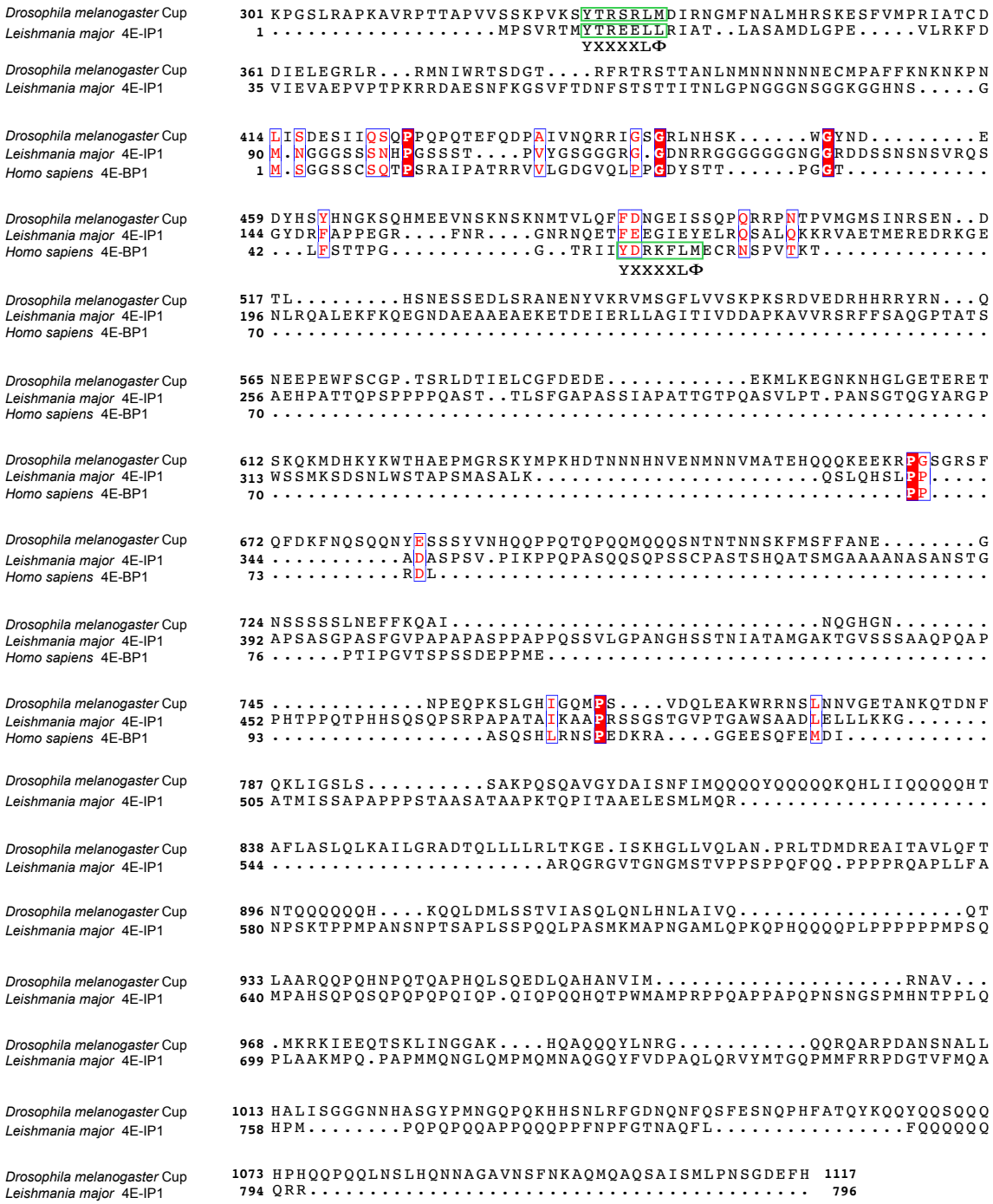
We performed yeast two-hybrid assays using the commercial GAL4 Two-Hybrid Phagemid Vector Kit (Stratagene) according to the manufacturer's instructions. We cloned the open reading frame of IF4E-1 into the GAL4-binding domain vector (pBD) using EcoRI and SalI sites. We cloned the open reading frame of Leish4E-IP1 into the GAL4 activation domain vector (pAD) through BamHI and XbaI sites. We generated point mutations in LeishIF4E-1 and Leish4E-IP1 by site directed mutagenesis. We co-transformed the yeast strain YRG-2 (Mata ura352 his3-200 ade2-101 lys2-801 trp1-901 leu2-3 112 gal4-542 gal80-538 LYS2::UASGAL1-TATA GAL1-HIS3 URA3::UASGAL4 17mers(x3) TATACYC1-lacZ) with the specific pAD and pBD constructs. We cultured the yeast transformants in Liquid SD-2 ($^{-}$Trp/$^{-}$Leu) medium at 30 °C overnight, diluted to a final concentration of $O.D_{600}$ of 0.15 and continued growth to an $O.D_{600}$ of 0.5. We then spotted yeast on SD-2 ($^{-}$Trp/$^{-}$Leu) and SD-3 ($^{-}$Trp/$^{-}$Leu/$^{-}$His) plates and grew them at 30 °C.

Figure S1

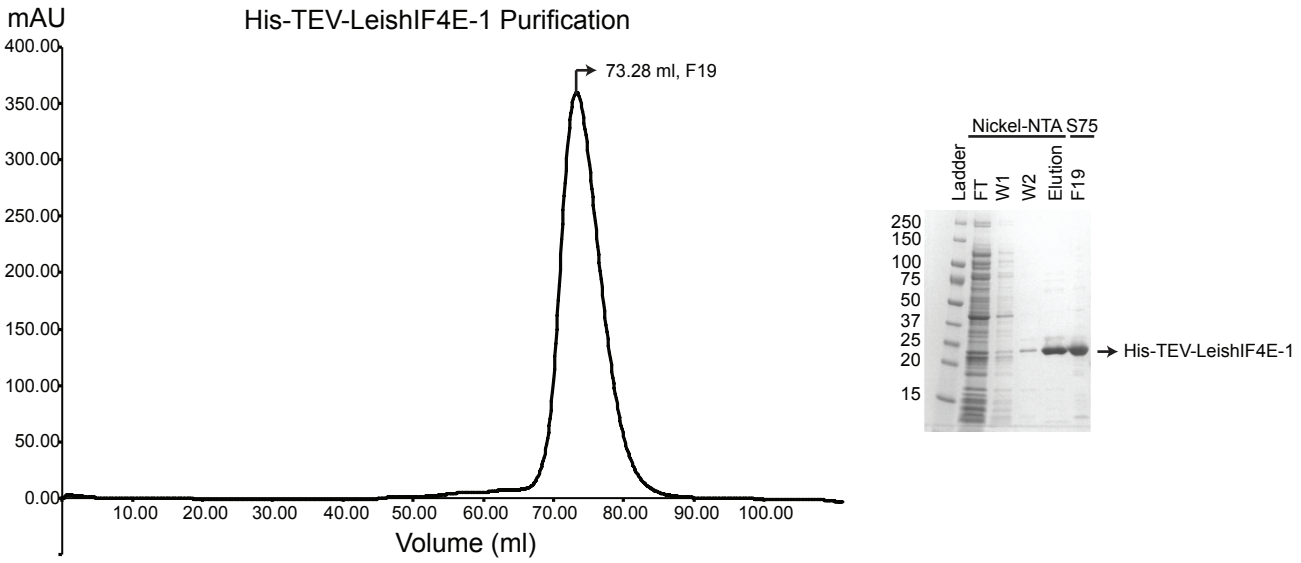
A


Homo sapiens IF4E
Mus musculus IF4E
Saccharomyces cerevisiae IF4E
Leishmania major IF4E-1
Leishmania major IF4E-2
Leishmania major IF4E-3
Leishmania major IF4E-4
Leishmania major IF4E-5
Leishmania major IF4E-6
β1
η1
β2
α1
η2
β3
β4
α2
η3
β5
β6
α3
β7
α4
β8

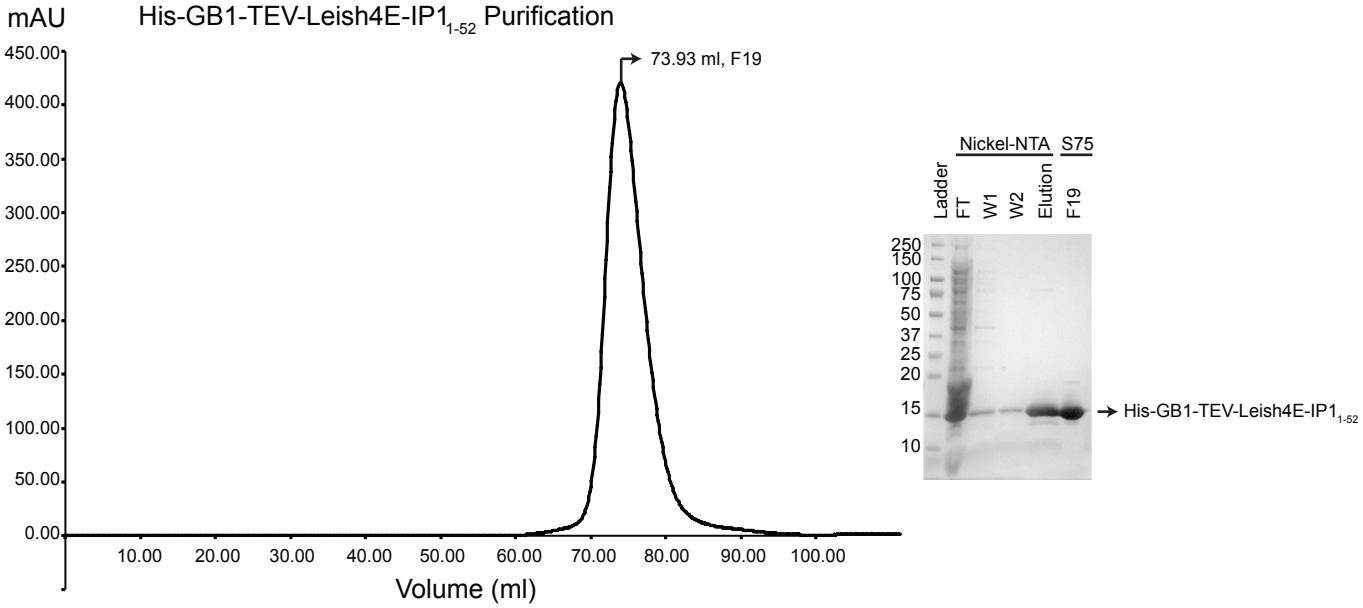
B


Drosophila melanogaster Cup
Leishmania major 4E-IP1
Homo sapiens 4E-BP1
YXXXXLΦ
YXXXXLΦ

C


mAU
His-TEV-LeishIF4E-1 Purification
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
73.28 ml, F19
10.00 20.00 30.00 40.00 50.00 60.00 70.00 80.00 90.00 100.00
Volume (ml)
Nickel-NTA
S75
Ladder
FT
W1
W2
Elution
F19
250
150
100
75
50
37
25
20
15
His-TEV-LeishIF4E-1

D


mAU
His-GB1-TEV-Leish4E-IP1$_{1-52}$ Purification
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
73.93 ml, F19
10.00 20.00 30.00 40.00 50.00 60.00 70.00 80.00 90.00 100.00
Volume (ml)
Nickel-NTA
S75
Ladder
FT
W1
W2
Elution
F19
250
150
100
75
50
37
25
20
15
10
His-GB1-TEV-Leish4E-IP1$_{1-52}$

E

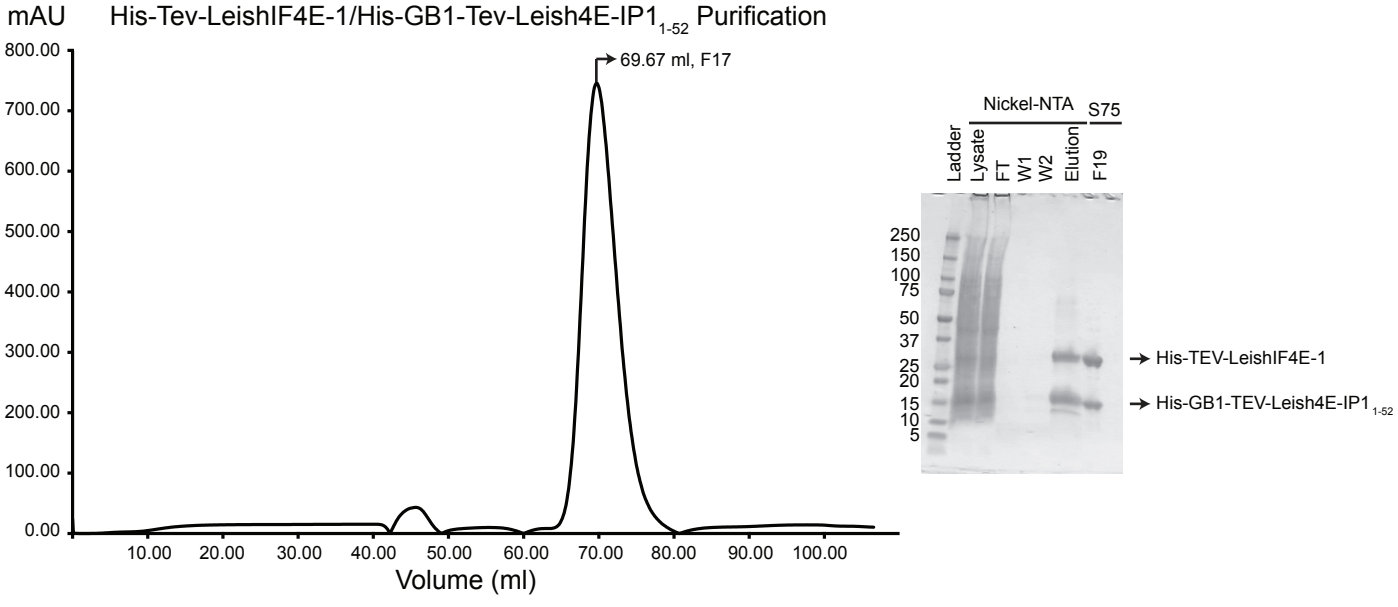

mAU
His-Tev-LeishIF4E-1/His-GB1-Tev-Leish4E-IP1$_{1-52}$ Purification
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
69.67 ml, F17
10.00 20.00 30.00 40.00 50.00 60.00 70.00 80.00 90.00 100.00
Volume (ml)
Nickel-NTA
S75
Ladder
Lysate
FT
W1
W2
Elution
F19
250
150
100
75
50
37
25
20
15
10
5
His-TEV-LeishIF4E-1
His-GB1-TEV-Leish4E-IP1$_{1-52}$

Figure S1. Leish(IF4E-1/4E-IP1$_{1-52}$) complex preparation, based on amino acid alignments and protein purification, related to Figure 1 and Figure 2

(A) Sequence alignment of LeishIF4E cap binding proteins. Sequence alignment was generated using Clustal Omega (2, 3) and is displayed using ESPript3 (4). White letters over a red background correspond to identical residues while red letters over a white background show conservation. Secondary structure information for the aligned sequences are indicated (α: alpha helices, η: 3_{10}-helix, β: beta-strands, TT: strict β-turns) and are rendered, respectively, as medium and large squiggles, and as arrows. Stars indicate conserved tryptophans from LeishIF4E-1 when compared to mammalian IF4E, while residues from LeishIF4E-1 involved in binding the cap are indicated by red dots. UniProt accession no.: *Homo sapiens* P06730, Mus musculus P63073, *Saccharomyces cerevisiae* P07260, *Leishmania major* IF4E-1 E9ADE1, IF4E-2 Q4QD60, IF4E-3 Q4Q813, IF4E-4 Q4Q7R3 IF4E-5 Q4Q217, IF4E-6 Q4Q9G7.

(B) Sequence alignment of LeishIF4E binding proteins. Sequence alignment was generated using Clustal Omega and is displayed using ESPript3 as described in (*A*). The consensus binding motif (YXXXXLΦ) is indicated and boxed in green for each IF4E binding protein. UniProt accession no: *Drosophila melanogaster* Cup Q9VMA3, *Leishmania major* 4E-IP1 E9AFM3, *Homo sapiens* 4E-BP1 Q13541.

(C) Purification of recombinant His-TEV-LeishIF4E-1 protein. His-TEV-LeishIF4E-1 was expressed in bacterial cells and purified over a nickel-NTA agarose affinity column followed by gel filtration chromatography using a Superdex 75 HiLoad 16/60 column (left panel). Several fractions were analyzed by SDS-gel and stained by Coomassie along the purification steps (right panel). The band corresponding to LeishIF4E-1 is indicated by an arrow. For nickel-NTA purification (nickel-NTA): FT: flow-through, W1: first wash, W2: second wash and Elution. For Superdex 75 purification (S75): F19: fraction 19.

(D) Purification of recombinant His-GB1-TEV-Leish4E-IP1$_{1-52}$. The purification scheme is as described in (*C*). The band corresponding to Leish4E-IP1 is indicated by an arrow.

(E) Purification of a recombinant Leish(IF4E-1/4E-IP1$_{1-52}$) complex. Both proteins were expressed in bacterial cells and pellets were mixed before purification over a nickel-NTA agarose affinity column. Proteins were further purified by gel filtration chromatography using a Superdex 75 HiLoad 16/60 column and fractions were analyzed by SDS-gel and stained by Coomassie along the purification steps, as described in (*C*). The bands corresponding to LeishIF4E-1 and Leish4E-IP1 within the complex are indicated by an arrow.

Figure S2

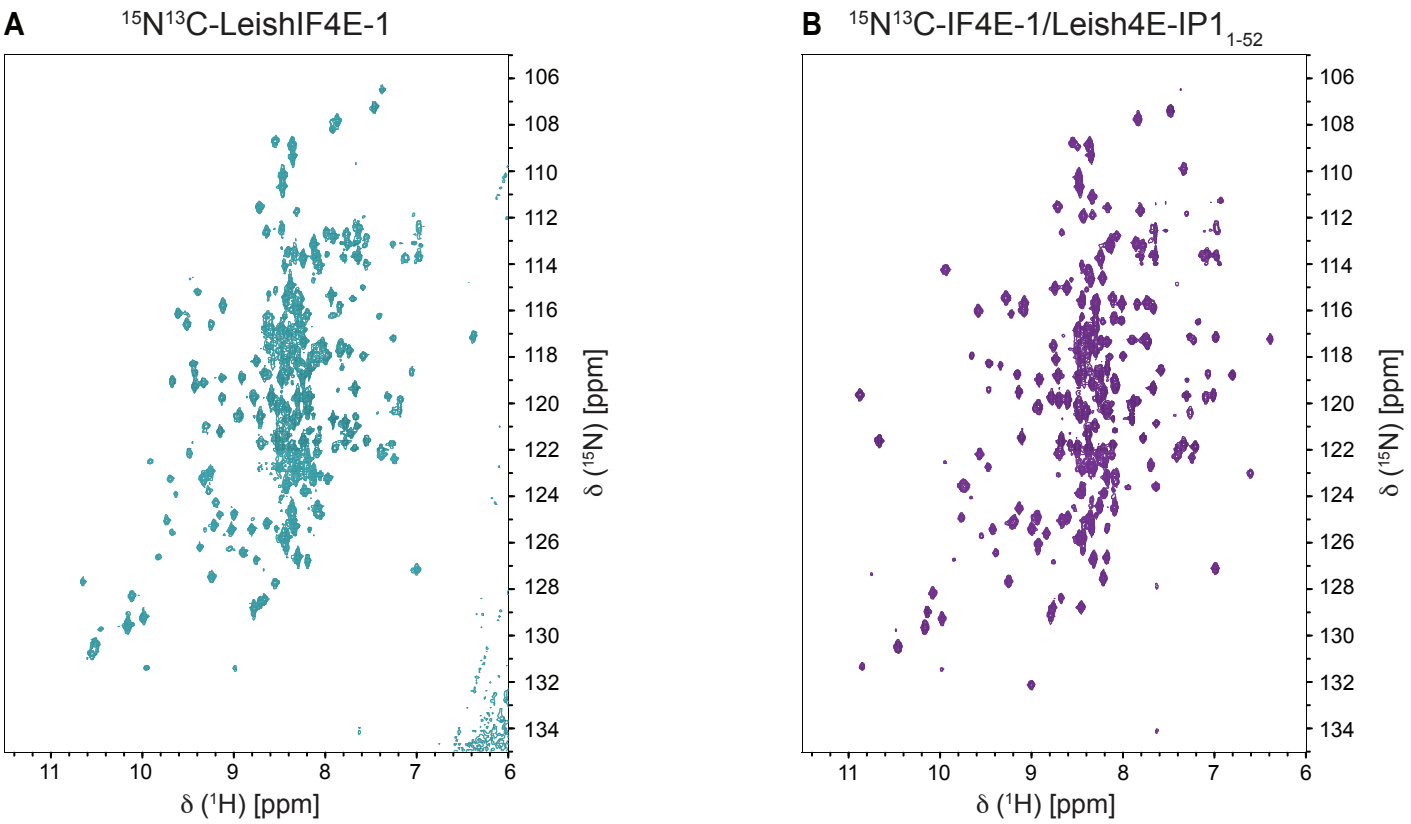

A
$^{15}N^{13}C$-LeishIF4E-1
B
$^{15}N^{13}C$-IF4E-1/Leish4E-IP1$_{1-52}$
δ (^{15}N) [ppm]
δ (^{1}H) [ppm]
106
108
110
112
114
116
118
120
122
124
126
128
130
132
134
11
10
9
8
7
6

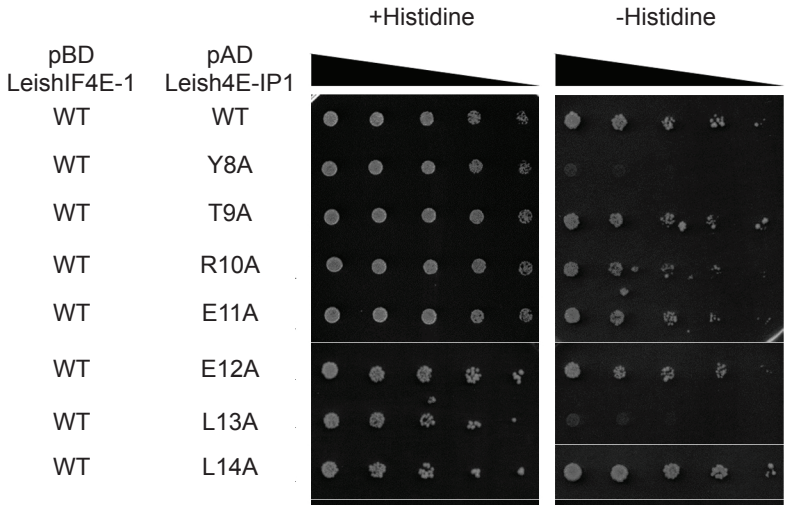
Figure S2. NMR spectroscopy of LeishIF4E-1 bound to a fragment of Leish4E-IP1, Related to Figure 1 and Figure 3

(A) ^{15}N-^{1}H-TROSY-HSQC spectrum of $^{15}N^{13}C$-LeishIF4E-1 acquired on a 600 MHz spectrometer.

(B) As described in (*A*), but where $^{15}N^{13}C$-LeishIF4E-1 is bound to unlabeled Leish4E-IP1$_{1\text{-}52}$ in an equimolar ratio.

Figure S3

A


+Histidine
-Histidine
pBD LeishIF4E-1
pAD Leish4E-IP1
WT WT
WT Y8A
WT T9A
WT R10A
WT E11A
WT E12A
WT L13A
WT L14A

B

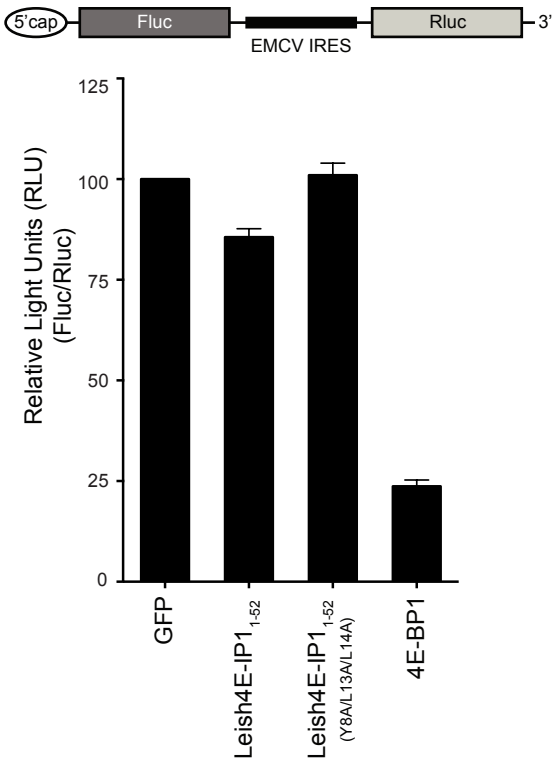
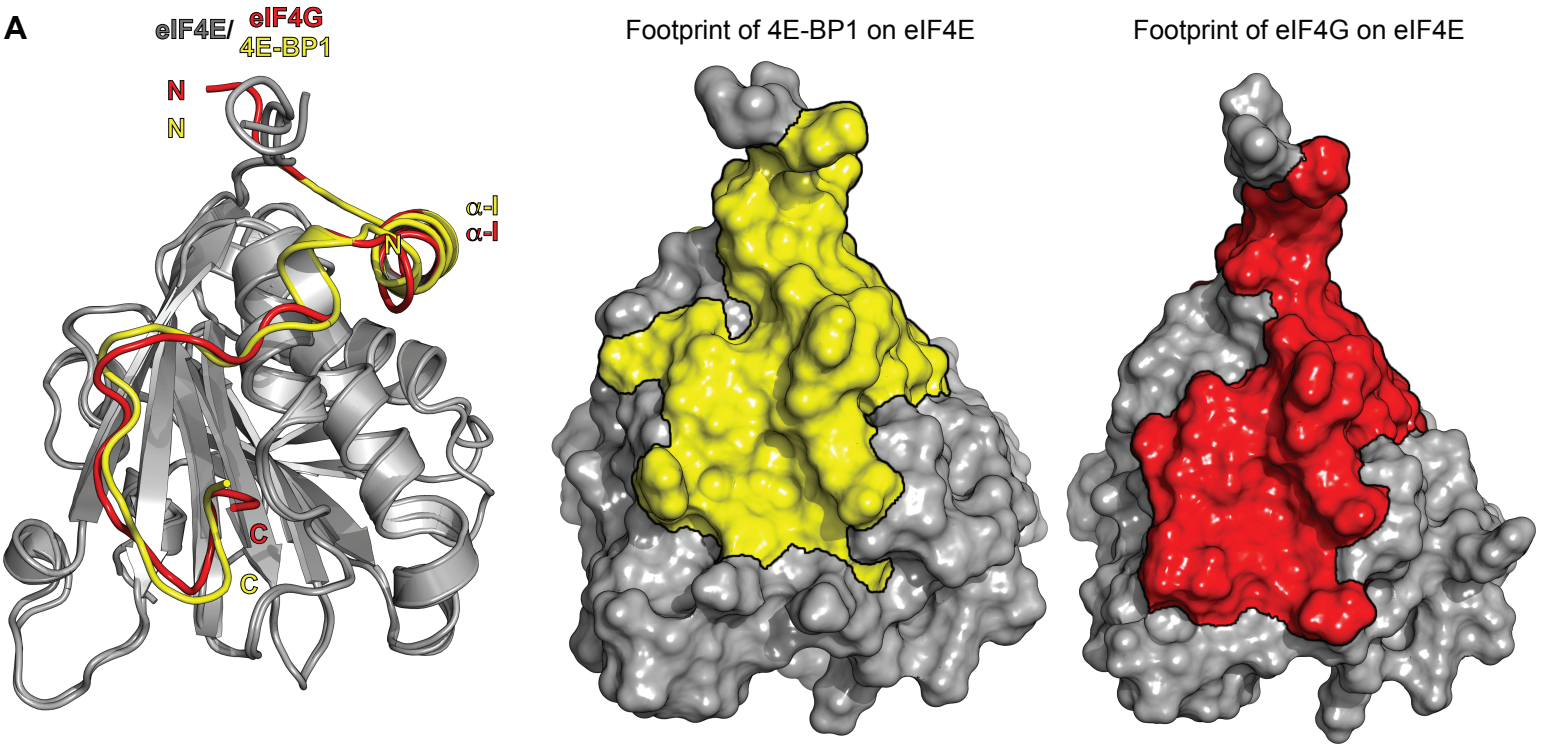

5'cap
Fluc
EMCV IRES
Rluc
3'
Relative Light Units (RLU) (Fluc/Rluc)
125
100
75
50
25
0
GFP
Leish4E-IP1 1-52
Leish4E-IP1 1-52 (Y8A/L13A/L14A)
4E-BP1

Figure S3. Effect of Leish4E-IP1 on mammalian translation, Related to Figure 2

(A) Yeast two-hybrid assay probing the Leish(IF4E-1/4E-IP1) interaction upon mutations in the consensus binding motif [$^{8}Y(X)_{4}L\Phi^{14}$]. LeishIF4E-1 was fused to the GAL4-binding domain vector (pBD) while Leish4E-IP1 was fused to the GAL4 activation domain vector (pAD). The yeast strain YRG-2 was co-transformed with specific pAD and pBD constructs.

(B) Upper panel: Diagram representing a dual-luciferase reporter system where an IRES (EMCV) is inserted between genes encoding the Firefly (Fluc) and the Renilla (Rluc) luciferase. Lower panel: Luciferase assay in which HEK293T cells were co-transfected with the dual luciferase reporter shown in the upper panel and with the plasmids encoding GFP, Leish4E-IP1$_{1\text{-}52}$-GFP, mutant Leish4E-IP1$_{1\text{-}52}$Y8A/L13A/L14A-GFP or with 4E-BP1-GFP. The results are presented as a percentage value when compared to the 100% value assigned to the GFP construct. The values are the means of at least three independent experiments, with bars representing the standard error on the means.

Figure S4


A
eIF4E/ eIF4G
4E-BP1
N
N
α-I
α-I
C
C
Footprint of 4E-BP1 on eIF4E
Footprint of eIF4G on eIF4E

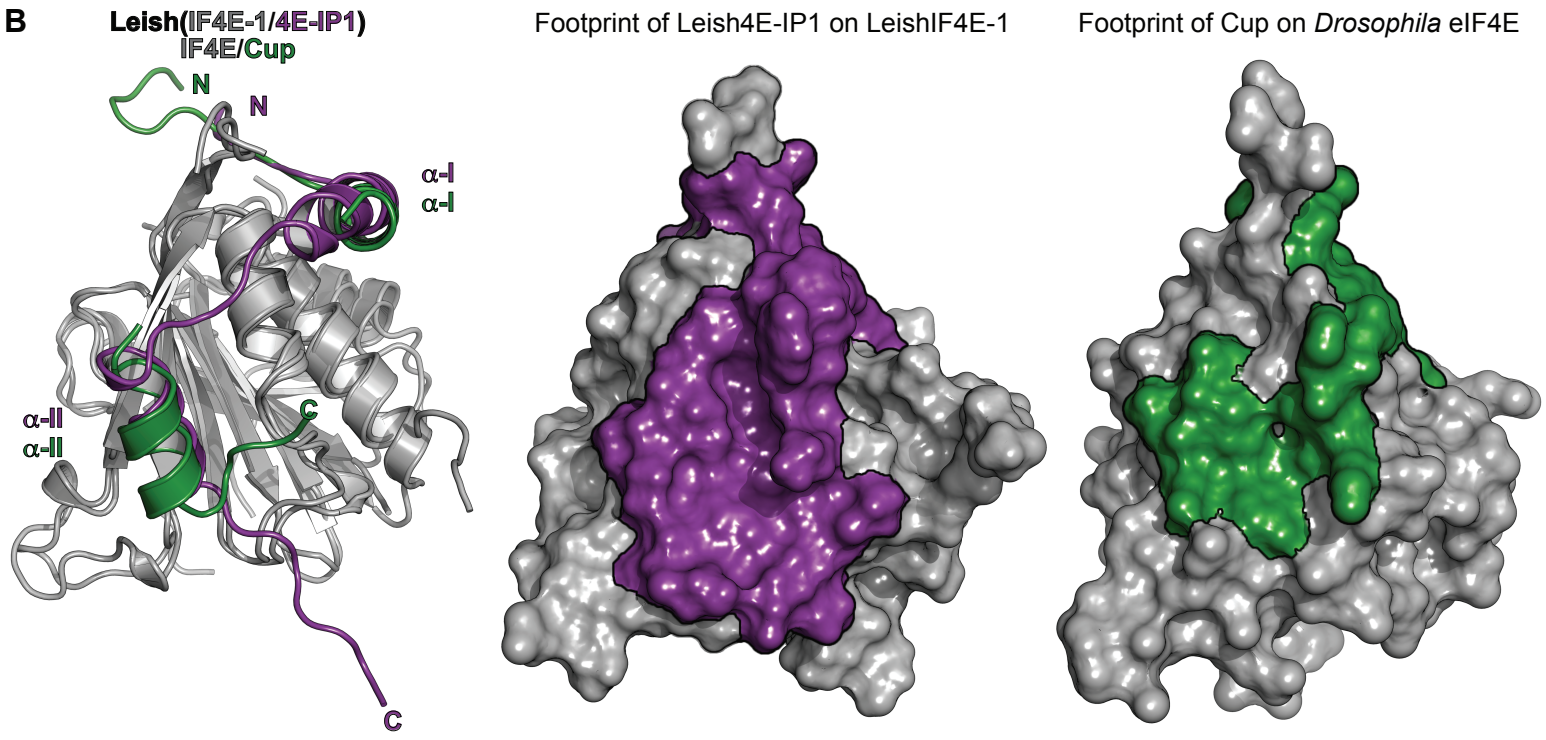

B
Leish(IF4E-1/4E-IP1)
IF4E/Cup
N
N
α-I
α-I
α-II
α-II
C
C
Footprint of Leish4E-IP1 on LeishIF4E-1
Footprint of Cup on *Drosophila* eIF4E

Figure S4. Footprint analysis of the cap-binding proteins on IF4E, related to Figure 3

(A) Left panel: Ribbon diagram of an overlay of eIF4E/eIF4G complex with eIF4E/4E-BP1, centered on helix α–II. eIF4E is colored in gray in both complexes while 4E-BP1 is represented in yellow (PDB ID: 5BXV) and eIF4G is shown in red (PDB ID: 5T46). The C and N termini are indicated for both proteins. The footprint analysis showed in the middle panel (4E-BP1), and the right panel (eIF4G) are colored accordingly and highlight interacting located within 4 Å to eIF4E.

(B) Left panel: As described in (*A*), except showing the binding interface of Leish4E-IP1 (purple, PDB ID: 5WB5) on LeishIF4E-1 (gray) from *Leishmania major* and Cup (green, PDB ID: 4AXG) on IF4E (gray) from *Drosophila melanogaster*. The footprint of Cup on IF4E is incomplete since the linker portion between helix α-I and helix α-II is disordered in the crystal structure.

Table S1: Data collection and refinement statistics (molecular replacement), Related to Figure 1 and 2

Leish(IF4E-1$_{12-210}$/4E-IP$_{14-43}$) complex	
Data collection	
Space group	$P6_222$
Cell dimensions	
a, b, c (Å)	68.22 68.22 220.20
α, β, γ (°)	90 90 120
Resolution (Å)	36.7 - 2.7 (2.796 - 2.700)*
R_{meas}	13.4 (47.7)
CC1/2	99.90
Average $I/\sigma I$	37.1 (14.68)*
Completeness (%)	99.77 (100)*
Number of reflections observed	584551
Number of unique reflections	9019
Refinement	
Resolution (Å)	36.7 - 2.7
No. Reflections	9013 (856)*
R_{work}/R_{free}	0.2396 (0.2894)*/0.2816 (0.3082)*
No. of atoms	1678
Protein	1650
Ligand/ions	7
Water	21
B-factors (Å^2)	
Protein	60.19
Ligand/ions	60.22
Solvent	37.4
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.49
Ramachandran favored (%)	97.97
Ramachandran allowed (%)	2.03
Ramachandran outliers (%)	0
Rotamer outliers (%)	0
Clashscore	3.02
PDB ID	5WB5

Complete datasets of five crystals were collected and scaled together to generate the Leish(IF4E-1/4E-IP1$_{1-52}$) model. *Values in parentheses are for the highest-resolution shell.

REFERENCES

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3. Goujon,M., McWilliam,H., Li,W., Valentin,F., Squizzato,S., Paern,J. and Lopez,R. (2010) A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res.*, **38**, W695–9.

4. Robert,X. and Gouet,P. (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.*, **42**, W320–4.