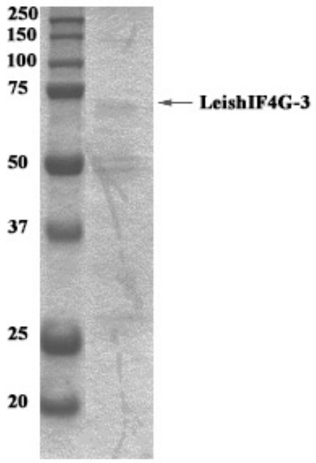
Yoffe et al 2009 - Evolutionary changes in the *Leishmania* eIF4F complex involve variations in the eIF4E-eIF4G interactions

SUPPLEMENTARY DATA

Supplementary Figure 1

A.



B. Protein = hypothetical protein, conserved [Leishmania major] |68125695|

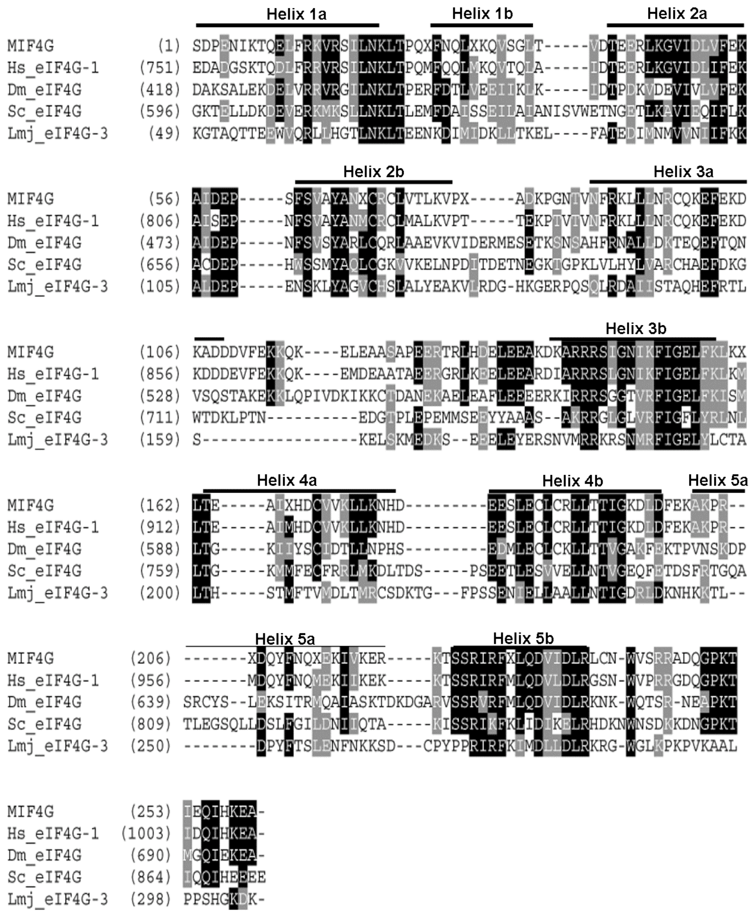
```
  1  MQFTVEQIRS VRNNYLEPPY PGFSLDEVVR RRRLTQTKLV RGENAWVAKG
 51  TAQTTEEWVQ RLLHGTLNKL TEENKDIMID KLLTKELFAT EDIMNMVVNI
101  IFKKALDEPE NSKLYAGVCH SLALYEAKVL RDGHKGERPQ SQLRDAIIST
151  AQHEFRTLSK ELSKMEDKSE EELEYERSNV MRRKRSNMRF IGELYLCTAL
201  THSTMFTVMD LTMRCSDKTG FPSSENIELL AALLNTIGDR LDKNHKKTLD
251  PYFTSLENFN KKSDCPYPPR IRFKIMDLLD LRKRGWGLKP KPVKAALPPS
301  HGKDKKYATA PPPKKGGRDQ GSAAAASKSW RDAATTKTGT AAAPAPSADK
351  GLNGRGTSSA APAANTAALS SSAASTAAAT APAGASFRDE ASPQVPLVKF
401  ELRVASMFQE WVADRTNDVI LHWVDQFNTC DRFFESESEL CVAVAQEVIH
451  SACTTTRKDA QREAFSFLVV GLYMIDTEVF DGFASALASA IEDGLLEDVP
501  KFGERFMSML RLTSTEQETR ADVYFDAANV LRLTYNRLES PDDSAVDTLM
551  SFWDRVPLPS TDEENMIRMD LDVVYSLCNP EGVQDGLEKL LSRIIHSMLQ
601  MQLMDAEVLD EFLCLDVEDG LCAKVIADYK ERFPK
```

MS analysis	Peptide	Coverage	score
#1	MQFTVEQIRS		63
	GTAQTTEEWVQRL		77
		3.5%	
#2	DAIISTAQHEFR	2%	96 (pep-miner)

Accession number in LeishDB – LmjF16.6060

Analysis of the 70 kDa protein eluted from m^7GTP-Sepharose. A. Coomassie staining of proteins that were eluted from a m^7GTP-Sepharose column, following their concentration by TCA. The ~70 kDa band that was excised and analyzed by Mass spectrometry is marked by an arrow. **B.** The amino acid coverage of peptides identified in independent Mass Spectrometry analysis is given in different colors (blue and red).

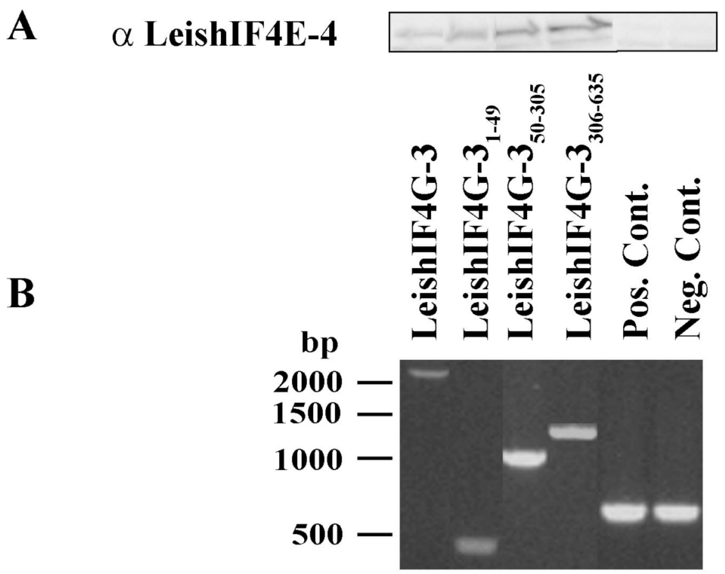
Supplementary Figure 2



```
MIF4G          (1) SDPENIKTQELFRKVRSILNKLTPQXFNQLXKQVSGLT-----VDTEERLKGVIDLVFEK
Hs_eIF4G-1   (751) EDADGSKTQDLFRRVRSILNKLTPQMFQQLMKQVTQLA-----IDTEERLKGVIDLIFEK
Dm_eIF4G     (418) DAKSALEKDELVRRVRGILNKLTPERFDTLVEEIIKLK-----IDTPDKVDEVIVLVFEK
Sc_eIF4G     (596) GKTELLDKDEVERKMKSLLNKLTLEMFDAISSEILAIANISVWETNGETLKAVIEQIFLK
Lmj_eIF4G-3   (49) KGTAQTTEEWVQRLLHGTLNKLTEENKDIMIDKLLTKEL----FATEDIMNMVVNIIFKK

MIF4G         (56) AIDEP-----SFSVAYANXCRCLVTLKVPX-----ADKPGNTVNFRKLLLNRCQKEFEKD
Hs_eIF4G-1   (806) AISEP-----NFSVAYANMCRCLMALKVPT-----TEKPTVTVNFRKLLLNRCQKEFEKD
Dm_eIF4G     (473) AIDEP-----NFSVSYARLCQRLAAEVKVIDERMESETKSNSAHFRNALLDKTEQEFTQN
Sc_eIF4G     (656) ACDEP-----HWSSMYAQLCGKVVKELNPDITDETNEGKIGPKLVLHYLVARCHAEFDKG
Lmj_eIF4G-3  (105) ALDEP-----ENSKLYAGVCHSLALYEAKVLRDG-HKGERPQSQLRDAIISTAQHEFRTL

MIF4G        (106) KADDDVFEKKQK----ELEAASAPEERTRLHDELEEAKDKARRRSIGNIKFIGELFKLKX
Hs_eIF4G-1   (856) KDDDEVFEKKQK----EMDEAATAEERGRLKEELEEARDIARRRSLGNIKFIGELFKLKM
Dm_eIF4G     (528) VSQSTAKEKKLQPIVDKIKKCTDANEKAELEAFLEEEERKIRRRSGGTVRFIGELFKISM
Sc_eIF4G     (711) WTDKLPTN----------EDGTPLEPEMMSEEYYAAAS--AKRRGLGLVRFIGFLYRLNL
Lmj_eIF4G-3  (159) S-----------------KELSKMEDKS--EEELEYERSNVMRRKRSNMRFIGELYLCTA

MIF4G        (162) LTE-----AIXHDCVVKLLKNHD---------EESLECLCRLLTTIGKDLDFEKAKPR--
Hs_eIF4G-1   (912) LTE-----AIMHDCVVKLLKNHD---------EESLECLCRLLTTIGKDLDFEKAKPR--
Dm_eIF4G     (588) LTG-----KIIYSCIDTLLNPHS---------EDMLECLCKLLTTVGAKFEKTPVNSKDP
Sc_eIF4G     (759) LTG-----KMMFECFRRLMKDLTDS-----PSEETLESVVELLNTVGEQFETDSFRTGQA
Lmj_eIF4G-3  (200) LTH-----STMFTVMDLTMRCSDKTG---FPSSENIELLAALLNTIGDRLDKNHKKTL--

MIF4G        (206) -------XDQYFNQXEKIVKER-----KTSSRIRFXLQDVIDLRLCN-WVSRRADQGPKT
Hs_eIF4G-1   (956) -------MDQYFNQMEKIIKEK-----KTSSRIRFMLQDVLDLRGSN-WVPRRGDQGPKT
Dm_eIF4G     (639) SRCYS--LEKSITRMQAIASKTDKDGARVSSRVRFMLQDVIDLRKNK-WQTSR-NEAPKT
Sc_eIF4G     (809) TLEGSQLLDSLFGILDNIIQTA-----KISSRIKFKLIDIKELRHDKNWNSDKKDNGPKT
Lmj_eIF4G-3  (250) --------DPYFTSLENFNKKSD---CPYPPRIRFKIMDLLDLRKRG-WGLKPKPVKAAL

MIF4G        (253) IEQIHKEA-
Hs_eIF4G-1  (1003) IDQIHKEA-
Dm_eIF4G     (690) MGQIEKEA-
Sc_eIF4G     (864) IQQIHEEEE
Lmj_eIF4G-3  (298) PPSHGKDK-
```

Sequence alignment of MIF4G domains. The amino acid sequences of MIF4G domains from different eIF4G proteins were subjected to multiple alignment using the Muscle algorithm. The predicted α-helical secondary structure elements (1-5, a and b) are indicated above the sequences. Accession numbers are: MIF4G, gi:13399859; eIF4G-1 of Human (Hs), Q04637; *Drosophila melanogaster* (Dm), NP_524640; *Saccharomyces cerevisiae* (Sc), Tif4631, NP_011678; and *Leishmania major* (Lmj) LeishIF4G-3, LmjF16.1600. Identical and similar residues are highlighted in black and gray, respectively.

Supplementary Figure 3



Genes encoding for *Leishmania* proteins are present in the transgenic yeast cells. The presence of the LeishIF4E-4 and LeishIF4G-3 was detected by Western analysis **(A)** or PCR **(B),** respectively**.**

Supplementary Table 1

Primers used for the cloning and site-directed mutagenesis. Small letters represent addition of restriction sites.

Leish4G-3 FwBamHI	cgggatccATGCAGTTCACCGTGGAGCA
Leish4G-3 RvStuI	aaggcctTTACTTGGGGAAGCGCTCCT
Leish4G-3 RvHindIII_NoStop	cccaagcttCTTGGGGAAGCGCTCCT
Leish4G-3 RvXbaFLAG	ctagtctagaCTATTTATCGTCATCGTCCTTGTAGTCCTTGGGGAAGCGCTCCT
Leish4G-3 147-129 StopRvEcoRI	ccggaattcTTACTTCGCAACCCAAGCATTC
Leish4G-3 148-166 FwBamHI	cgggatccGGCACCGCTCAGACGAC
Leish4G-3 915- 898 StopRvEcoRI	ccggaattcTTATTTATCCTTGCCGTGGCT
Leish4G-3 916-934 FwBamHI	cgggatccAAGTACGCCACTGCCC
Leish4G-3 Δ23-29 Fw	GAGCCGCCGTACCCGGGCCGACGCCGTCGCTTGAC
Leish4G-3 Δ23-29 Rv	GTCAAGCGACGGCGTCGGCCCGGGTACGGCGGCTC
Leish4G-3 Δ20-25 Fw	TACCTTGAGCCGCCGGAAGTGGTGCGACGC
Leish4G-3 Δ20-25 Rv	GCGTCGCACCACTTCCGGCGGCTCAAGGTA
Leish4G-3 Y20A Fw	ACCTTGAGCCGCCGGCCCCGGGCTTTTCTCT
Leish4G-3 Y20A Rv	AGAGAAAAGCCCGGGGCCGGCGGCTCAAGGT
Leish4G-3 Y20F Fw	ACCTTGAGCCGCCGTTCCCGGGCTTTTCTCT
Leish4G-3 Y20F Rv	AGAGAAAAGCCCGGGAACGGCGGCTCAAGGT
Leish4G-3 P21A Fw	TTGAGCCGCCGTACGCGGGCTTTTCTCTGGA
Leish4G-3 P21A Rv	TCCAGAGAAAAGCCCGCGTACGGCGGCTCAA
Leish4G-3 G22A Fw	AGCCGCCGTACCCGGCCTTTTCTCTGGATGA
Leish4G-3 G22A Rv	TCATCCAGAGAAAAGGCCGGGTACGGCGGCT
Leish4G-3 F23A Fw	CCGTACCCGGGCGCTTCTCTGGATGAAGTGG
Leish4G-3 F23A Rv	CCACTTCATCCAGAGAAGCGCCCGGGTACGG
Leish4G-3 S24A Fw	CGTACCCGGGCTTTGCTCTGGATGAAGTGGT
Leish4G-3 S24A Rv	ACCACTTCATCCAGAGCAAAGCCCGGGTACG
Leish4G-3 L25A Fw	ACCCGGGCTTTTCTGCGGATGAAGTGGTGCG
Leish4G-3 L25A Rv	CGCACCACTTCATCCGCAGAAAAGCCCGGGT
Leish4G-3 D26A Fw	CGGGCTTTTCTCTGGCTGAAGTGGTGCGACG
Leish4G-3 D26A Rv	CGTCGCACCACTTCAGCCAGAGAAAAGCCCG
Leish4G-3 E27A Fw	GCTTTTCTCTGGATGCAGTGGTGCGACGCCG
Leish4G-3 E27A Rv	CGGCGTCGCACCACTGCATCCAGAGAAAAGC
Leish4G-3 V29F Fw	CTCTGGATGAAGTGTTTCGACGCCGTCGCTT
Leish4G-3 V29F Rv	AAGCGACGGCGTCGAAACACTTCATCCAGAG
Leish4E-1 FwBamHI	cgcggatccATGTCAGCCCCGTCTTCAGTT
Leish4E-1 RvXba_No STOP	gctctagaAGACGCCTCGCCGTGCTT
Leish4E-4 FwBamHI	cgcggatccATGAACCCCAACGCCACGGA
Leish4E-4 RvXba_No STOP	gctctagaGTAGCGCCGACGGTTCTT
Leish4E-4 FwEcoRI	ggaattcATGAACCCCAACGCCACGGA
Leish4E-4 RvSalI	acgcgtcgacTTAGTAGCGCCGACGGTTCTT
pAD 5'	Agggatgtttaataccactac
pAD 3'	Gcacagttgaagtgaacttgc