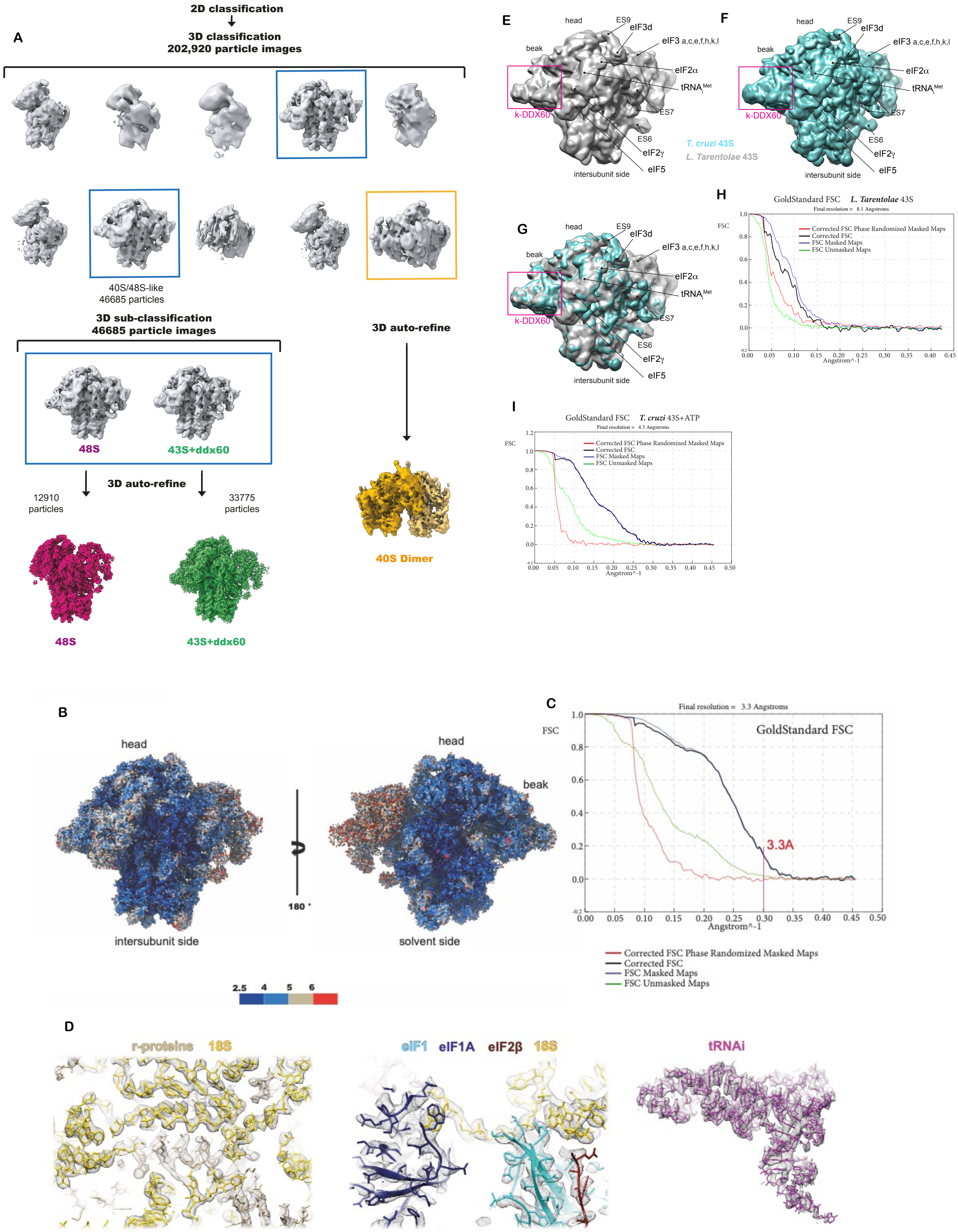
Cell Reports, Volume *33*

Supplemental Information

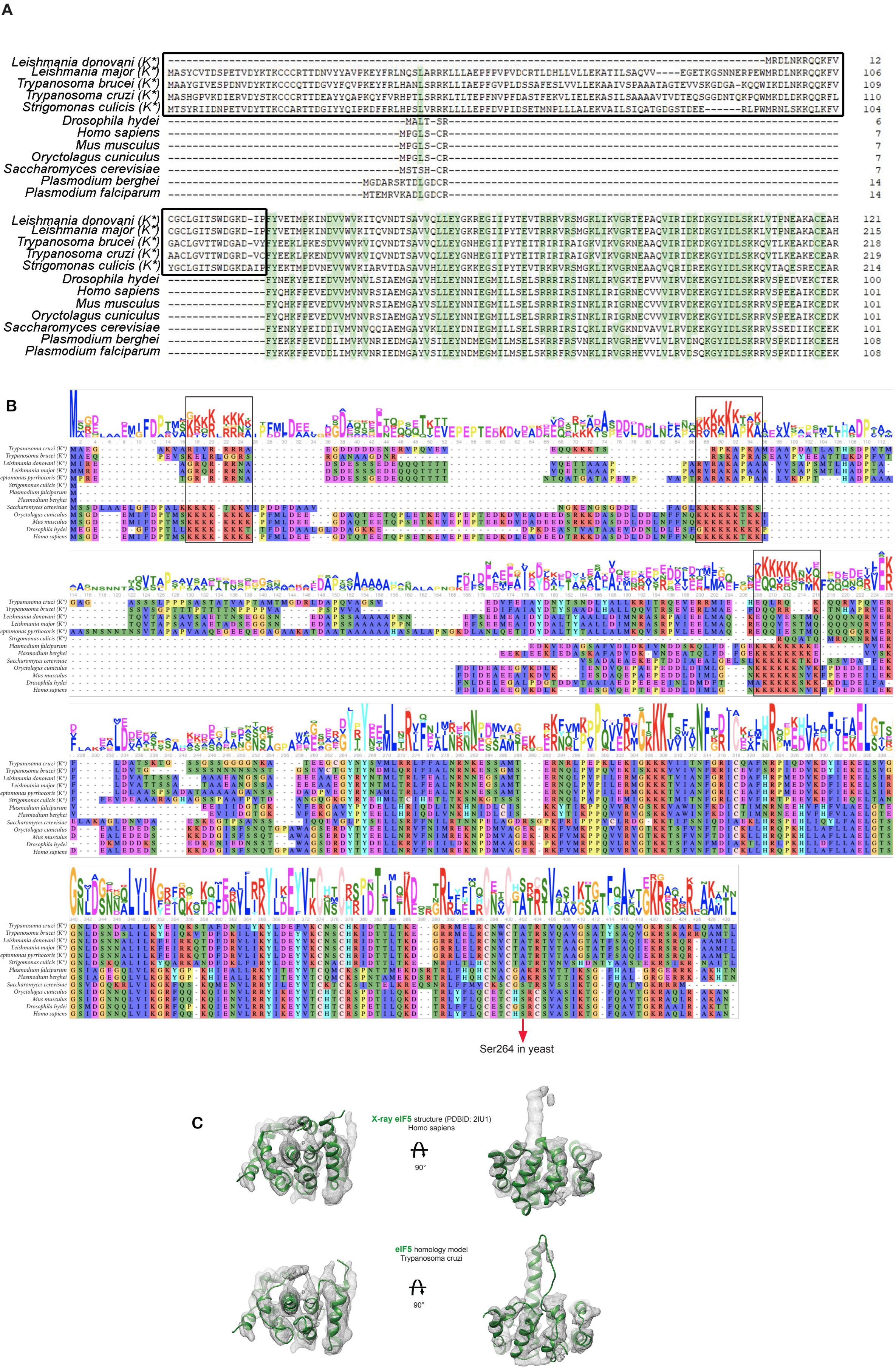
Structural Differences in Translation Initiation between Pathogenic Trypanosomatids and Their Mammalian Hosts

Anthony Bochler, Jailson Brito Querido, Terezie Prilepskaja, Heddy Soufari, Angelita Simonetti, Mayara Lucia Del Cistia, Lauriane Kuhn, Aline Rimoldi Ribeiro, Leoš Shivaya Valášek, and Yaser Hashem

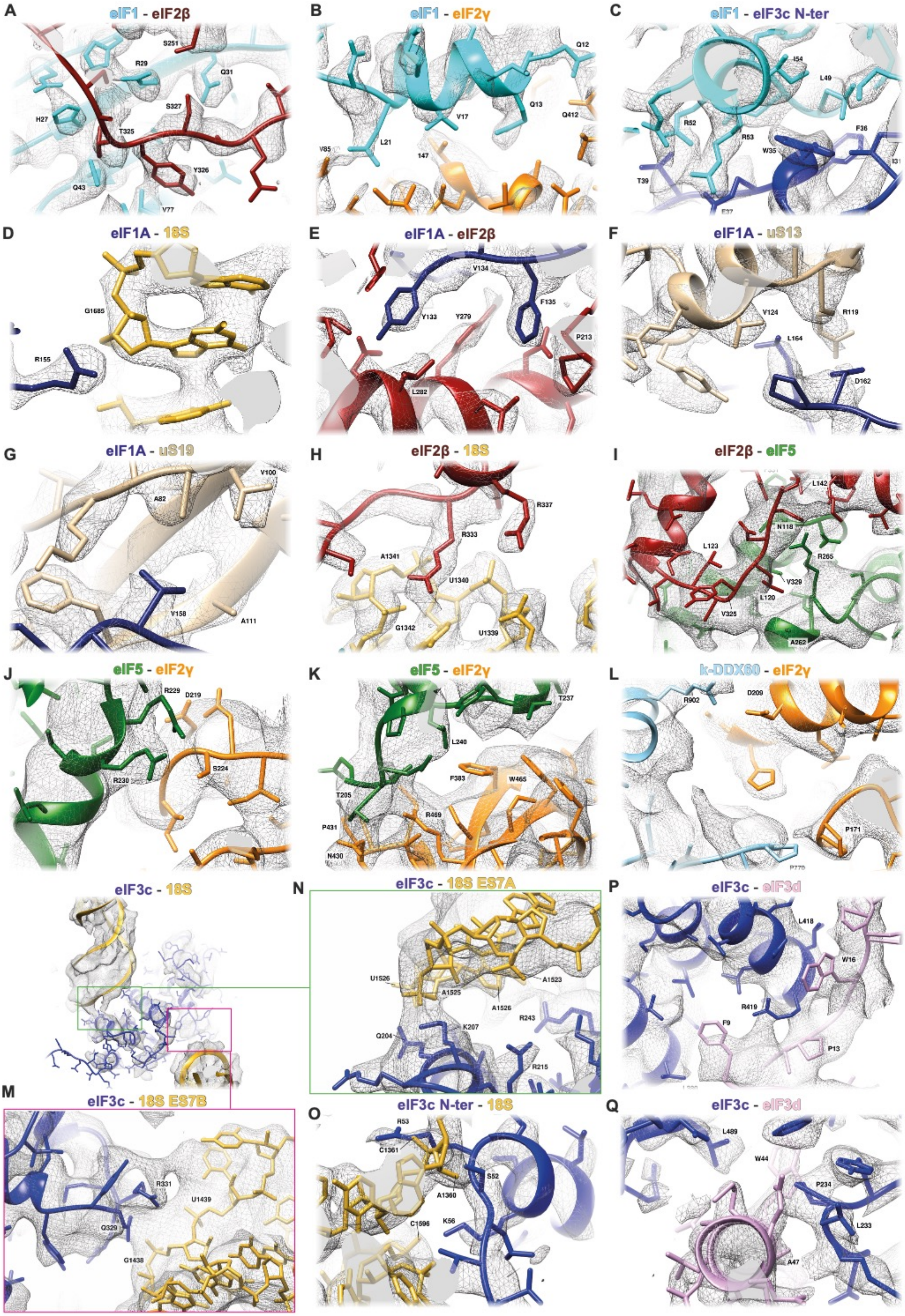
SUPPLEMENTARY FIGURES


A
2D classification
3D classification
202,920 particle images
40S/48S-like
46685 particles
3D sub-classification
46685 particle images
48S
43S+ddx60
3D auto-refine
12910
particles
33775
particles
48S
43S+ddx60
3D auto-refine
40S Dimer
E
head
ES9
eIF3d
beak
eIF3 a,c,e,f,h,k,l
eIF2α
tRNA$_i^{Met}$
k-DDX60
ES7
ES6
eIF2γ
eIF5
intersubunit side
T. cruzi 43S
L. Tarentolae 43S
F
head
ES9
eIF3d
beak
eIF3 a,c,e,f,h,k,l
eIF2α
tRNA$_i^{Met}$
k-DDX60
ES7
ES6
eIF2γ
eIF5
intersubunit side
G
head
ES9
eIF3d
beak
eIF3 a,c,e,f,h,k,l
eIF2α
tRNA$_i^{Met}$
k-DDX60
ES7
ES6
eIF2γ
eIF5
intersubunit side
H
GoldStandard FSC L. Tarentolae 43S
Final resolution = 8.1 Angstroms
FSC
Corrected FSC Phase Randomized Masked Maps
Corrected FSC
FSC Masked Maps
FSC Unmasked Maps
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
Angstrom^-1
I
GoldStandard FSC T. cruzi 43S+ATP
Final resolution = 4.3 Angstroms
FSC
Corrected FSC Phase Randomized Masked Maps
Corrected FSC
FSC Masked Maps
FSC Unmasked Maps
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
Angstrom^-1
B
head
intersubunit side
180 °
head
beak
solvent side
2.5
4
5
6
C
Final resolution = 3.3 Angstroms
GoldStandard FSC
FSC
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
3.3A
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
Angstrom^-1
Corrected FSC Phase Randomized Masked Maps
Corrected FSC
FSC Masked Maps
FSC Unmasked Maps
D
r-proteins
18S
eIF1
eIF1A
eIF2β
18S
tRNAi

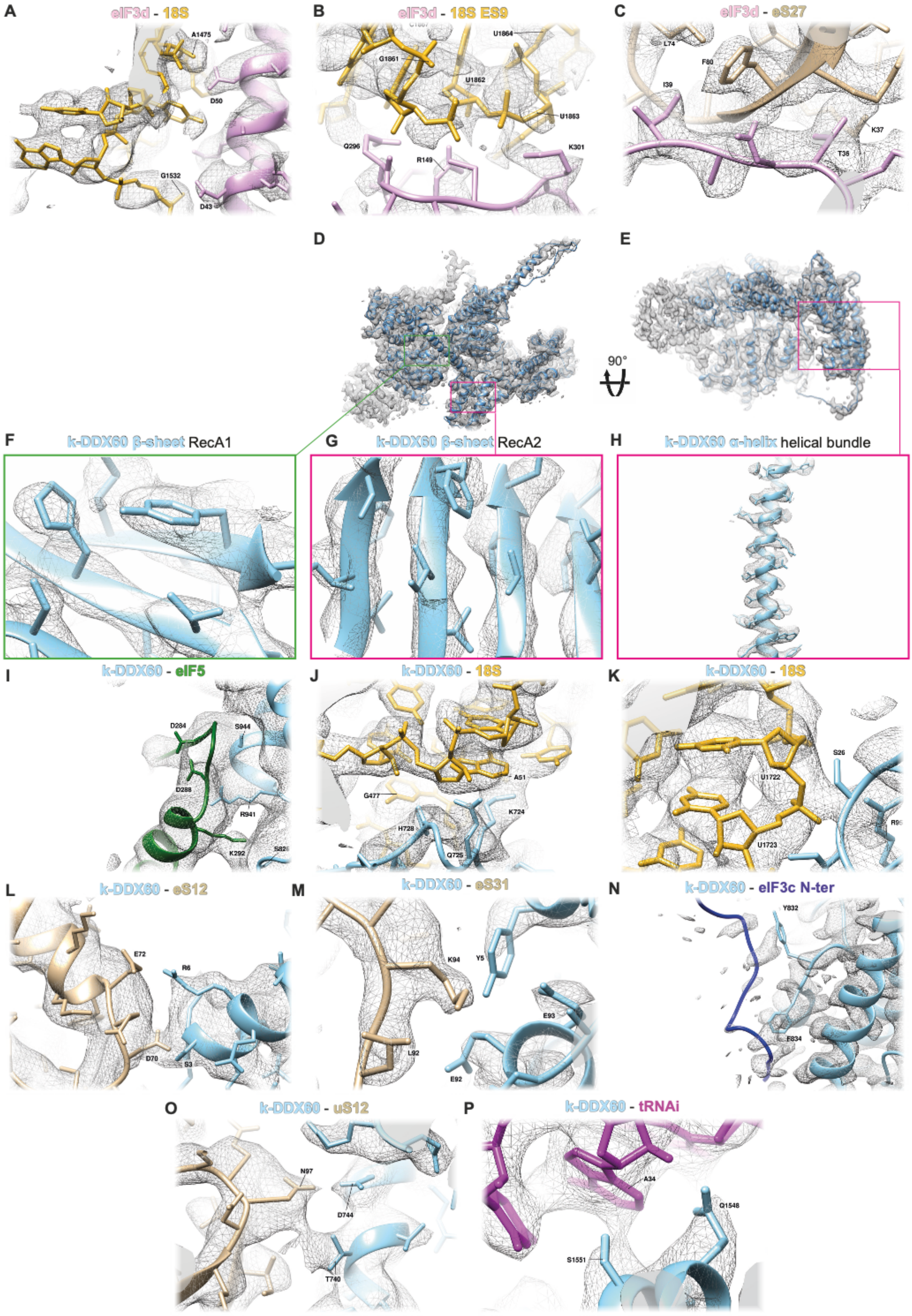
Supplementary Figure 1. Cryo-EM particle sorting and refinement of the 43S PIC complexes from *T. cruzi* and *L. tarentolae* and their resolutions, Related to Figure 1. (**A**) 2D classification of the 43S PIC particles yielded ~200 000 40S-like particles from the *T. cruzi* dataset, after which a run of 3D classification (10 classes) was performed. (**B**) The local resolution of the 43S class varies mainly on eIF3 (ranging from ~3 to ~6 Å), while is varies less on the rest of the structure (ranging from ~2.5 to ~3.5 Å for the 40S, k-DDX60, eIFs 1, 1A and 2b, and from ~3 to ~5 Å for eIFs 2a, 2g and 5). (**C**) The average resolution was measured after applying a soft-edge mask of the 43S PIC shape filtered to 15Å and extended by 3 pixels. (**D**) Blow ups on several features of the complex counting 40S rRNA/r-proteins (left), 18S rRNA interaction with eIF 1 and 1A (middle) and the initiator $\mathrm{tRNA^{Met}}$ (right), fitted in their corresponding densities. (**E**) Cryo-EM reconstructions of the *L. tarentolae* 43S PIC. (**F**) Cryo-EM reconstructions of the *T. cruzi* 43S PIC filtered at 8Å. (**G**) Superimposition of (E) and (F). (**H**) Average resolution (8.1Å) of the *L. tarentolae* 43S PIC reconstruction. (**I**) Average resolution (4.3Å) of the cryo-EM reconstruction from the *T. cruzi* 43S complexes supplemented with ATP.

A
Leishmania donovani (K*) --MRDLNKRQQKFV 12
Leishmania major (K*) MASYCVTDSPETVDYKTKCCCRTTDNVYYAVPKEYFRLNQSLARRKLLLAEPFPVPVDCRTLDHLLVLLEKATILSAQVV----EGETKGSNNERPEWMRDLNKRQQKFV 106
Trypanosoma brucei (K*) MAAYGIVESPDNVDYKTKCCCQTTDGVYFQVPKEYFRLHANLSRRKLLIAEPFGVPLDSSAFESLVVLLEKAAIVSPAAATAGTEVVSKGDGA-KQQWIKDLNKRQQKFV 109
Trypanosoma cruzi (K*) MASHGPVKDIERVDYSTKCCCRTTDEAYYQAPKQYFVRHPTLSRRKLTLTEPFNVPFDASTFEKVLIELEKASILSAAAAVTVDTEQSGGDNTQKPQWMKDLDKRQQRFL 110
Strigomonas culicis (K*) MTSYRIIDNPETVDYTTKCCARTTDGIYYQIPKDFFRLHPSLVRRKLLLEPFDVPIDSETMNPLLLALEKVAILSIQATGDGSTDEE------RLPWMRNLSKKQLKFV 104
Drosophila hydei ---------------------------------------MALT-SR-- 6
Homo sapiens --------------------------------------MPGLS-CR-- 7
Mus musculus --------------------------------------MPGLS-CR-- 7
Oryctolagus cuniculus --------------------------------------MPGLS-CR-- 7
Saccharomyces cerevisiae --------------------------------------MSTSH-CR-- 7
Plasmodium berghei --------------------------------MGDARSKTDLGDCR-- 14
Plasmodium falciparum --------------------------------MTEMRVKADLGDCR-- 14
Leishmania donovani (K*) CGCLGITSWDGKD-IPFYVETMPKINDVVWVKITQVNDTSAVVQLLEYGKREGIIPYTEVTRRRVRSMGKLIKVGRTEPAQVIRIDKDKGYIDLSKKLVTPNEAKACEAH 121
Leishmania major (K*) CGCLGITSWDGKD-IPFYVETMPKINDVVWVKITQVNDTSAVVQLLEYGKREGIIPYTEVTRRRVRSMGKLIKVGRTEPAQVIRIDKDKGYIDLSKKLVTPNEAKACEAH 215
Trypanosoma brucei (K*) GACLGVTTWDGAD-VYFYEEKLPKESDVVWVKVIQVNDTSAVVQLLEYGNHEGIIPYTEITRIRIRAIGKVIKVGKNEAAQVIRIDKDKGYIDLSKKQVTLKEAKDCEAR 218
Trypanosoma cruzi (K*) AACLGVTTWDGRD-VCFYEEKLPKENDVVWVKVIQVNDTSAVVQLLEYGNHEGIIPYTEITRIRIRAIGKVIKVGRNEAAQVIRIDKEKGYIDLSKKQVTLKEAKECEAR 219
Strigomonas culicis (K*) YGCLGITSWDGKDAIPFYEKTMPDVNEVVWVKIARVTDASAVVHLLEYGKKEGSIPYTEVTRKRVRSMGKLIKVGRNEAAQVQRIDREKGYIDLSKKQVTAQESRECEAR 214
Drosophila hydei ----------------FYNEKYPEIEDVVMVNVLSIAEMGAYVHLLEYNNIEGMILLSELSRRRIRSINKLIRVGKTEPVVVIRVDKEKGYIDLSKRRVSPEDVEKCTER 100
Homo sapiens ----------------FYQHKFPEVEDVVMVNVRSIAEMGAYVSLLEYNNIEGMILLSELSRRRIRSINKLIRIGRNECVVVIRVDKEKGYIDLSKRRVSPEEAIKCEDK 101
Mus musculus ----------------FYQHKFPEVEDVVMVNVRSIAEMGAYVSLLEYNNIEGMILLSELSRRRIRSINKLIRIGRNECVVVIRVDKEKGYIDLSKRRVSPEEAIKCEDK 101
Oryctolagus cuniculus ----------------FYQHKFPEVEDVVMVNVRSIAEMGAYVSLLEYNNIEGMILLSELSRRRIRSINKLIRIGRNECVVVIRVDKEKGYIDLSKRRVSPEEAIKCEDK 101
Saccharomyces cerevisiae ----------------FYENKYPEIDDIVMVNVQQIAEMGAYVKLLEYDNIEGMILLSELSRRRIRSIQKLIRVGKNDVAVVLRVDKEKGYIDLSKRRVSSEDIIKCEEK 101
Plasmodium berghei ----------------FYEKKFPEVDDLIMVKVNRIEDMGAYVSILEYNDMEGMILMSELSKRRFRSVNKLIRVGRHEVVLVLRVDNQKGYIDLSKRRVSPKDIIKCEEH 108
Plasmodium falciparum ----------------FYKKKFPEVDDLIMVKVNRIEDMGAYVSILEYNDMEGMILMSELSKRRFRSVNKLIRVGRHEVVLVLRVDSQKGYIDLSKRRVSPKDIIKCEEK 108
B
Trypanosoma cruzi (K*)
Trypanosoma brucei (K*)
Leishmania donovani (K*)
Leishmania major (K*)
Leptomonas pyrrhocoris (K*)
Strigomonas culicis (K*)
Plasmodium falciparum
Plasmodium berghei
Saccharomyces cerevisiae
Oryctolagus cuniculus
Mus musculus
Drosophila hydei
Homo sapiens
Ser264 in yeast
C
X-ray eIF5 structure (PDBID: 2IU1)
Homo sapiens
90°
eIF5 homology model
Trypanosoma cruzi
90°

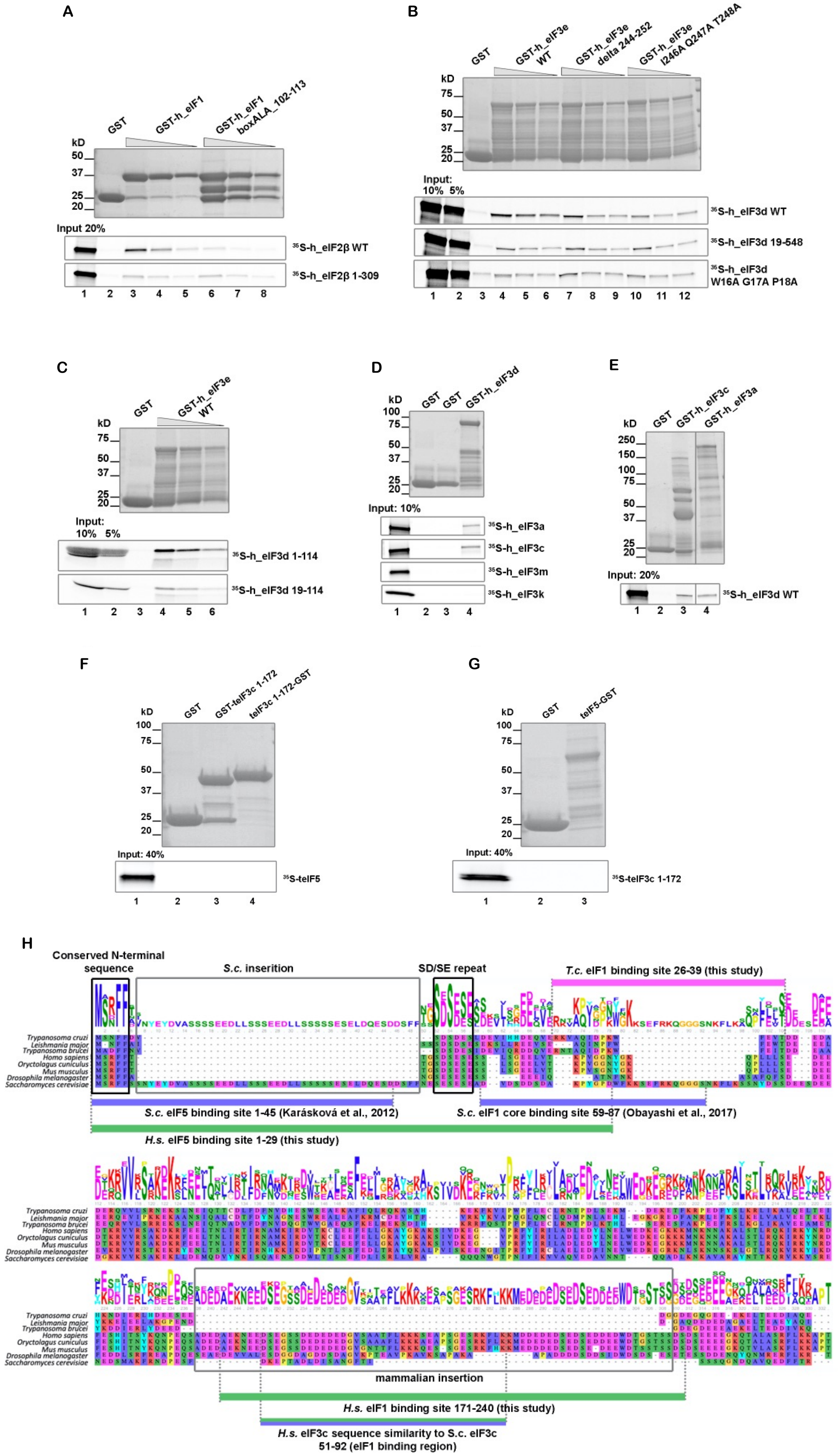
Supplementary Figure 2. Multiple sequence alignment of the eIF2α NTD and eIF2β among eukaryotes, and eIF5 CTD structure, Related to Figure 2. (**A**) Protein sequence alignment of eIF2α from various eukaryotic organisms was generated by Clone Manger (MultiWay, scoring matrix: Blosum 62). The Kinetoplastida order species are labeled with K*. The kinetoplastidian-specific eIF2α N-terminal domain insertion is marked with a black box. Areas of high matches (60%) are shaded in green. The individual species with the NCBI Reference Sequence numbers or TriTrypDB numbers are as follows: [*Trypanosoma cruzi*] PWV18423.1, [*Trypanosoma brucei*] Tb927.3.2900, [*Leishmania donovani*] AAQ02666.1, [*Leishmania major*] LmjF.03.0980, [*Strigomonas culicis*] EPY26930.1, *[Plasmodium falciparum* NF54] PKC42156.1, [*Plasmodium berghei* ANKA] VUC53995.1, [*Saccharomyces cerevisiae*] ONH75775.1, [*Oryctolagus cuniculus*] XP_002719561.1, [*Mus musculus*] NP_080390.1, [*Drosophila hydei*] XP_023166950.2, [*Homo sapiens*] NP_004085.1. (**B**) Protein sequence alignment of eIF2β protein from various eukaryotic organisms. The Kinetoplastida order species are labeled with K*. Consensus is expressed as a sequence logo. The black boxes mark three conserved poly-lysine stretches (dubbed K-boxes) K1, K2 and K3. (**C**) Rigid-body fittings of the crystal structure of the human eIF5 CTD in the corresponding *T. cruzi* 43S PIC density (up) and its *T. cruzi* eIF5 CTD homology model in that same density (bottom).


A
eIF1 - eIF2β
S251
R29
Q31
S327
H27
T325
Y326
Q43
V77
B
eIF1 - eIF2γ
Q12
V17
Q13
Q412
L21
V85
I47
C
eIF1 - eIF3c N-ter
I54
L49
R52
R53
F36
W35
I31
T39
E37
D
eIF1A - 18S
G1685
R155
E
eIF1A - eIF2β
V134
Y133
Y279
F135
P213
L282
F
eIF1A - uS13
V124
R119
L164
D162
G
eIF1A - uS19
V100
A82
V158
A111
H
eIF2β - 18S
R337
R333
A1341
U1340
G1342
U1339
I
eIF2β - eIF5
L142
N118
L123
R265
V329
L120
V325
A262
J
eIF5 - eIF2γ
R229
D219
R230
S224
K
eIF5 - eIF2γ
T237
L240
F383
W465
T205
R469
P431
N430
L
k-DDX60 - eIF2γ
R902
D209
P171
P770
eIF3c - 18S
N
eIF3c - 18S ES7A
U1526
A1525
A1523
A1526
R243
Q204
K207
R215
P
eIF3c - eIF3d
L418
W16
R419
F9
P13
M
eIF3c - 18S ES7B
R331
U1439
Q329
G1438
O
eIF3c N-ter - 18S
R53
C1361
S52
A1360
C1596
K56
Q
eIF3c - eIF3d
L489
W44
P234
L233
A47

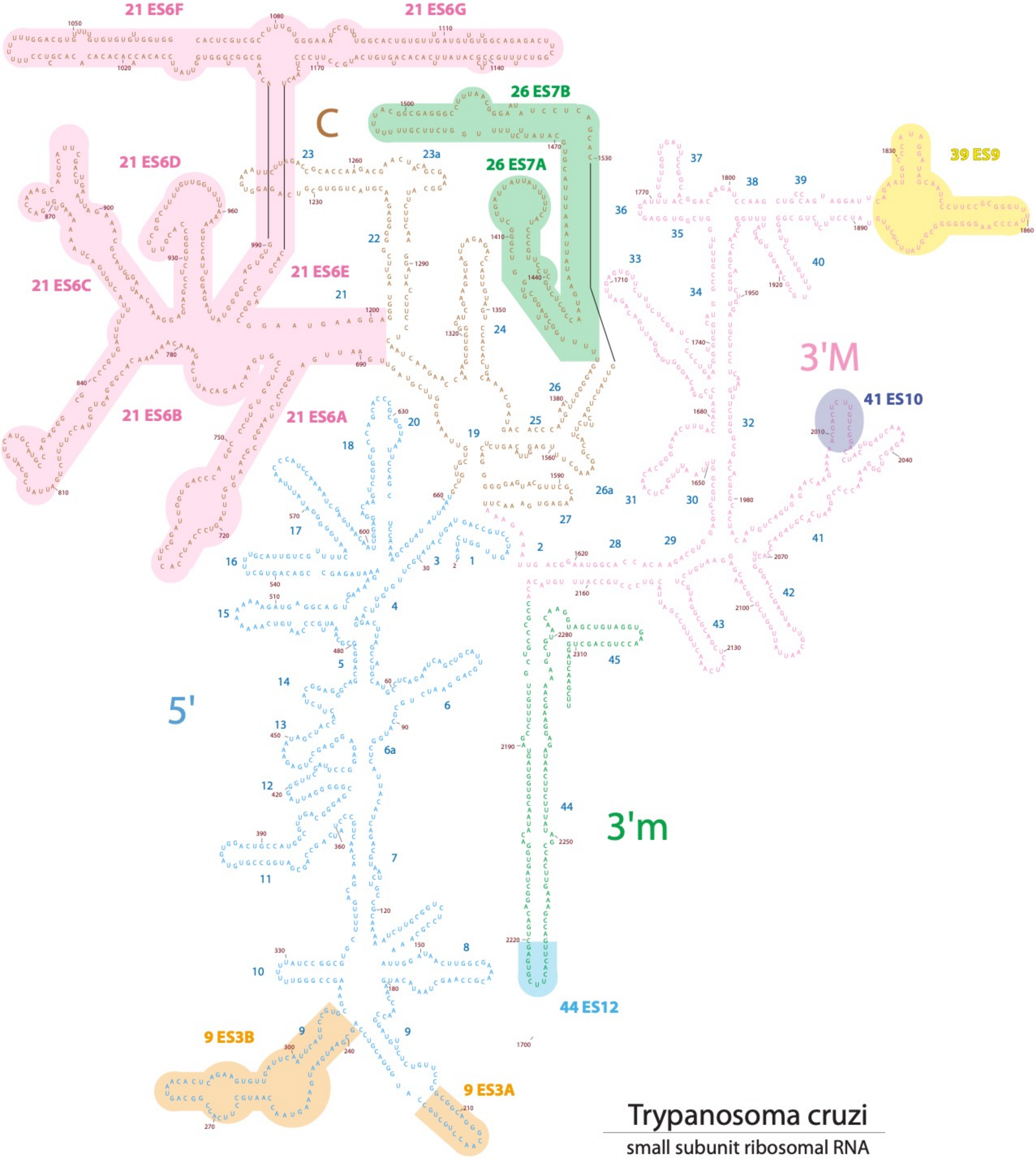
Supplementary Figure 3. Novel interactions between several eIFs, r-proteins and 18S rRNA, fitted in their corresponding densities, Related to Figures 2 and 3. (**A**) eIF1 with eIF2β. (**B**) eIF1 N-ter tail with eIF2γ. (**C**) eIF1 with eIF3c N-ter. (**D**) eIF1A with the 18S. (**E**) eIF1A with eIF2β. (**F**) eIF1A with uS13. (**G**) eIF1A with uS19. (**H**) eIF2β with the 18S. (**I**) eIF2β with eIF5 CTD. (**J** and **K**) eIF5 CTD with eIF2γ. (**L**) eIF2γ with k-DDX60. (**M**, **N** and **O**) 18S with eIF3c. (**P** and **Q**) eIF3c and eIF3d subunits.


A
eIF3d - 18S
A1475
D50
G1532
D43
B
eIF3d - 18S ES9
C1867
U1864
G1861
U1862
U1863
Q296
R149
K301
C
eIF3d - eS27
L74
F80
I39
K37
T36
D
E
90°
F
k-DDX60 β-sheet RecA1
G
k-DDX60 β-sheet RecA2
H
k-DDX60 α-helix helical bundle
I
k-DDX60 - eIF5
D284
S944
D288
R941
K292
J
k-DDX60 - 18S
A51
G477
K724
H728
Q725
K
k-DDX60 - 18S
S26
U1722
U1723
L
k-DDX60 - eS12
E72
R6
D70
S3
M
k-DDX60 - eS31
K94
Y5
E93
L92
E92
N
k-DDX60 - eIF3c N-ter
Y832
F834
O
k-DDX60 - uS12
N97
D744
T740
P
k-DDX60 - tRNAi
A34
Q1548
S1551

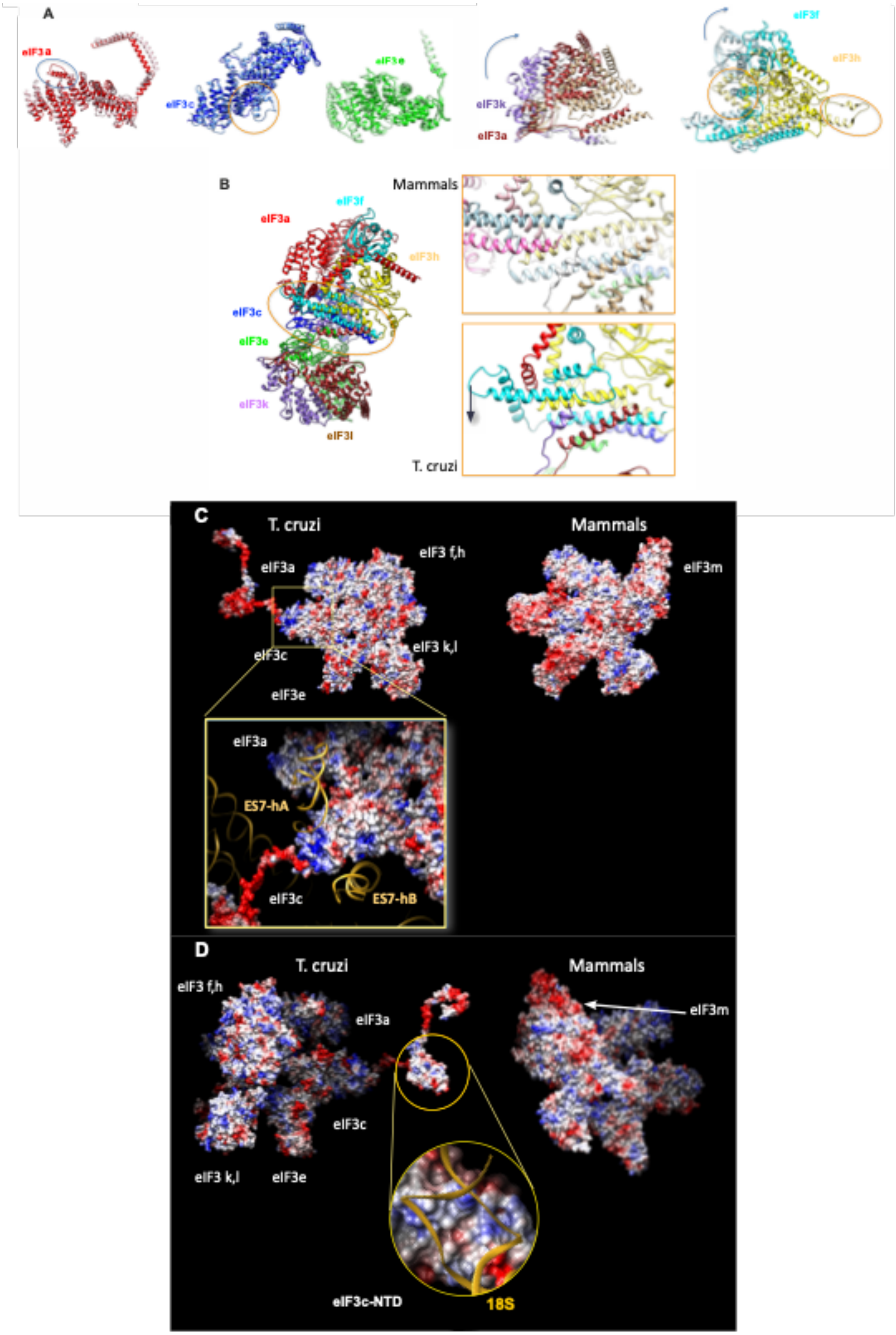
Supplementary Figure 4. Interactions of eIF3d and k-DDX60 with eIFs, r-proteins and 18S rRNA, fitted in their corresponding densities, Related to Figures 2 and 3. (**A** and **B**) eIF3d with the 18S. (**C**) eIF3d with eS27. (**D** and **E**) k-DDX60 in its corresponding density, viewed from two orientations. (**F**, **G** and **H**) Blow ups on two β-sheets from helicase RecA domains and one buried α-helix from a helical bundle from K-DDX60. Interactions of k-DDX60 with eIF5 (**I**), 18S (**J** and **K**), eS12 (**L**), eS31 (**M**), eIF3c N-ter (**N**), uS12 (**O**) and the initiator $tRNA^{Met}$ (**P**).


A
GST
GST-h_eIF1
GST-h_eIF1 boxALA_102-113
kD
50
37
25
20
Input 20%
35S-h_eIF2β WT
35S-h_eIF2β 1-309
1 2 3 4 5 6 7 8
B
GST
GST-h_eIF3e WT
GST-h_eIF3e delta 244-252
GST-h_eIF3e I246A Q247A T248A
kD
75
50
37
25
20
Input: 10% 5%
35S-h_eIF3d WT
35S-h_eIF3d 19-548
35S-h_eIF3d W16A G17A P18A
1 2 3 4 5 6 7 8 9 10 11 12
C
GST
GST-h_eIF3e WT
kD
75
50
37
25
20
Input: 10% 5%
35S-h_eIF3d 1-114
35S-h_eIF3d 19-114
1 2 3 4 5 6
D
GST
GST
GST-h_eIF3d
kD
100
75
50
37
25
20
Input: 10%
35S-h_eIF3a
35S-h_eIF3c
35S-h_eIF3m
35S-h_eIF3k
1 2 3 4
E
GST
GST-h_eIF3c
GST-h_eIF3a
kD
250
150
100
75
50
37
25
20
Input: 20%
35S-h_eIF3d WT
1 2 3 4
F
GST
GST-teIF3c 1-172
teIF3c 1-172-GST
kD
100
75
50
37
25
20
Input: 40%
35S-teIF5
1 2 3 4
G
GST
teIF5-GST
kD
100
75
50
37
25
20
Input: 40%
35S-teIF3c 1-172
1 2 3
H
Conserved N-terminal sequence
S.c. inserition
SD/SE repeat
T.c. eIF1 binding site 26-39 (this study)
Trypanosoma cruzi
Leishmania major
Trypanosoma brucei
Homo sapiens
Oryctolagus cuniculus
Mus musculus
Drosophila melanogaster
Saccharomyces cerevisiae
S.c. eIF5 binding site 1-45 (Karásková et al., 2012)
S.c. eIF1 core binding site 59-87 (Obayashi et al., 2017)
H.s. eIF5 binding site 1-29 (this study)
mammalian insertion
H.s. eIF1 binding site 171-240 (this study)
H.s. eIF3c sequence similarity to S.c. eIF3c 51-92 (eIF1 binding region)

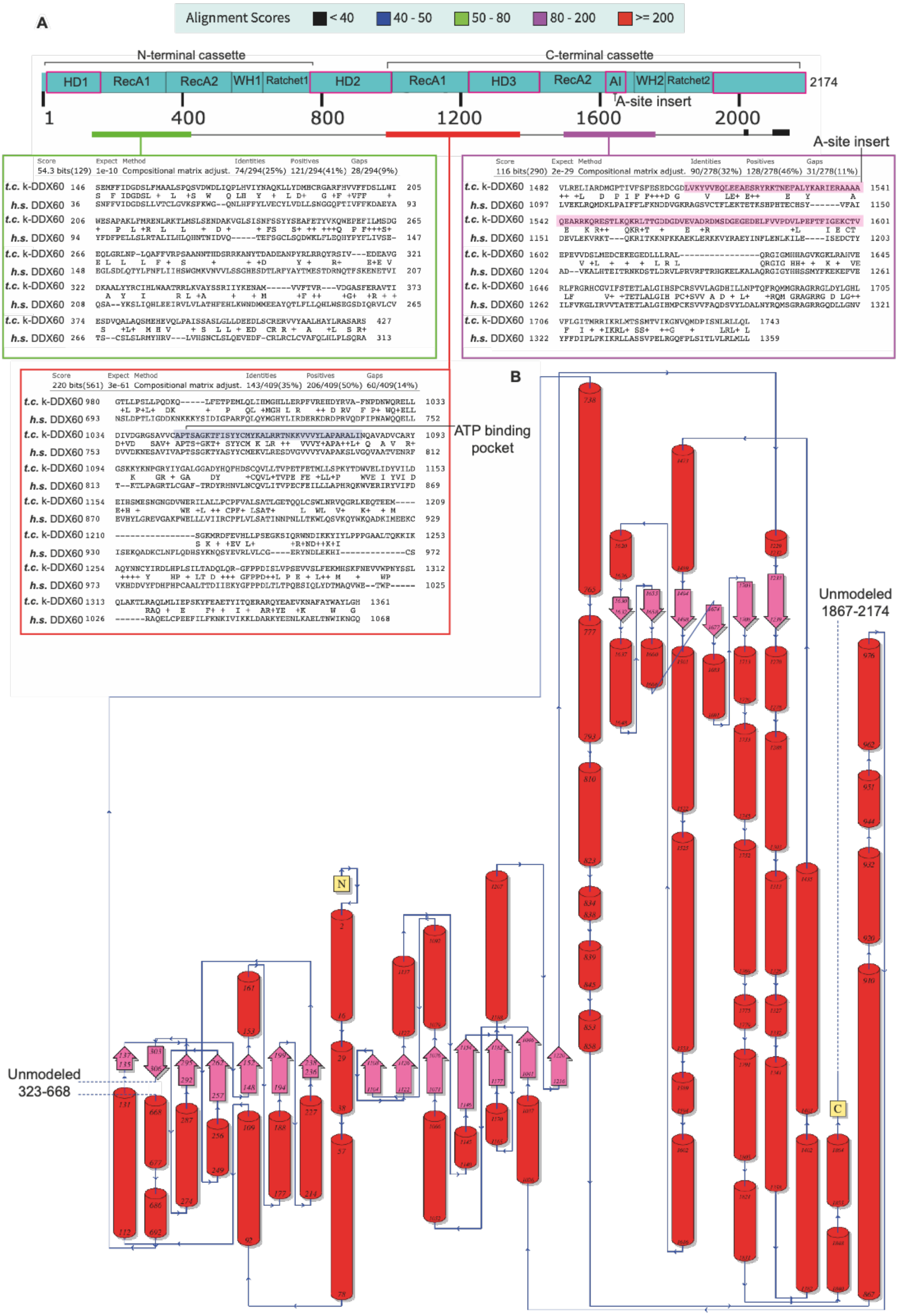
Supplementary Figure 5. *In vitro* analysis of eIF3 intersubunit interactions , Related to Figures 2 and 3. (**A**) *In vitro* protein-protein binding analysis of the interaction between the *in vitro* translated human ^{35}S-labeled eIF2β and its C-terminal truncation (eIF2β 1-309) against wild type eIF1 or its mutated variant (eIF1-boxAla-102-113; residues 102-113 substituted with a stretch of alanines) fused to GST. *In vitro* translated proteins were tested for binding with three different dilutions of individual GST-fusion proteins. Lane 1 contains 20% of input amounts of *in vitro*-translated proteins added to each reaction. (**B**) Same as in (A) except that binding between the human wild type eIF3d subunit, its N-terminally truncated form (19-548), and its mutated variant (W16A G17A P18A) against the human wild type eIF3e subunit, or its inner deletion (delta 244-252), or its mutated variant (I246A Q247A T248A) fused to GST was analyzed. Lanes 1 and 2 show 10% and 5% input, respectively. Quantification was performed by the Quantity One software (see Fig. 3J.) (**C**) Same as in (A) except that binding between truncations of the human eIF3d subunit (1-114 and 19-114) and eIF3e fused to GST was analyzed. Quantification is presented in Fig. 3K. (**D**) *In vitro* protein-protein binding analysis of ^{35}S-labeled eIF3a, eIF3c, eIF3k and eIF3m subunits against eIF3d fused to GST. Lane 1 shows 10% input. (**E**) *In vitro* protein-protein binding analysis of human ^{35}S-labeled eIF3d against eIF3c and eIF3a subunits fused to GST. Lane 1 shows 20% input. (**F**) *In vitro* protein-protein binding analysis of the interaction between *T. cruzi* eIF5 and the eIF3c-NTD (residues 1-172) fused with GST either at its N or C terminus. (**G**) Binding analysis of the interaction between *T.cruzi* the eIF3c-NTD (residues 1-172) and eIF5-GST. (**H**) Multiple protein alignment of the N-terminal domain of the eIF3c subunit from indicated species with a consensus expressed as a sequence logo. Specific sequence features mentioned in the main text are boxed. Positions of eIF1- and eIF5-binding sites in the eIF3c-NTD of the selected species identified by us and others are marked by thick lines under or above the alignment; color-coding is as follows: *T.c. – Trypanosoma cruzi* in pink, *S.c. – Saccharomyces cerevisiae* in purple, and *H.s. – Homo sapiens* in green.



Supplementary Figure 6. ***T. cruzi*** **18S rRNA, Related to Figures 4 and 5.** 2D diagram of the T. *cruzi* 18S rRNA. The largest and more relevant expansion segments are highlighted in colored backgrounds.


A
eIF3a
eIF3c
eIF3e
eIF3k
eIF3a
eIF3f
eIF3h
B
Mammals
eIF3f
eIF3a
eIF3h
eIF3c
eIF3e
eIF3k
eIF3l
T. cruzi
C
T. cruzi
eIF3 f,h
eIF3a
eIF3c
eIF3 k,l
eIF3e
Mammals
eIF3m
eIF3a
ES7-hA
eIF3c
ES7-hB
D
T. cruzi
eIF3 f,h
eIF3a
eIF3c
eIF3 k,l
eIF3e
Mammals
eIF3m
eIF3c-NTD
18S

Supplementary Figure 7. Charge surface analysis of the *T.cruzi* and mammalian eIF3 structures, Related to Figures 3. (A) Overlay of mammalian and kinetoplastidian structures of individual eIF3 subunits with marked structural differences. The *T. cruzi* structures are depicted in dark and mammalian in light color shades. Curved arrows indicate the direction of *T. cruzi* eIF3 subunits structural rearrangement compared to their mammalian counterparts. Colored ovals highlight marked structural differences between *T. cruzi* and mammalian eIF3 subunits. **(B)** Cartoon representation of the eIF3 atomic model showing the eIF3 helical bundle in mammals (upper panel) and in *T. cruzi* (lower panel). Dark arrow indicates the shift of a helix from eIF3f in *T. cruzi* to compensate for the absence of eIF3m. **(C)** Surface representation of the *T. cruzi* (left) and mammalian (right) eIF3 structure seen from the 40S platform side. Lower panel: close-up view of *T.cruzi* eIF3c and its interaction with the ES7^s helix A and helix B. Model is color-coded according to the electrostatic potential – negative in red and positive in blue. **(D)** Surface representation of the *T. cruzi* (left) and mammalian (right) eIF3 structure seen from the 40S solvent side. Lower panel: close-up view of the *T.cruzi* eIF3c-NTD and its interaction with 18S RNA.



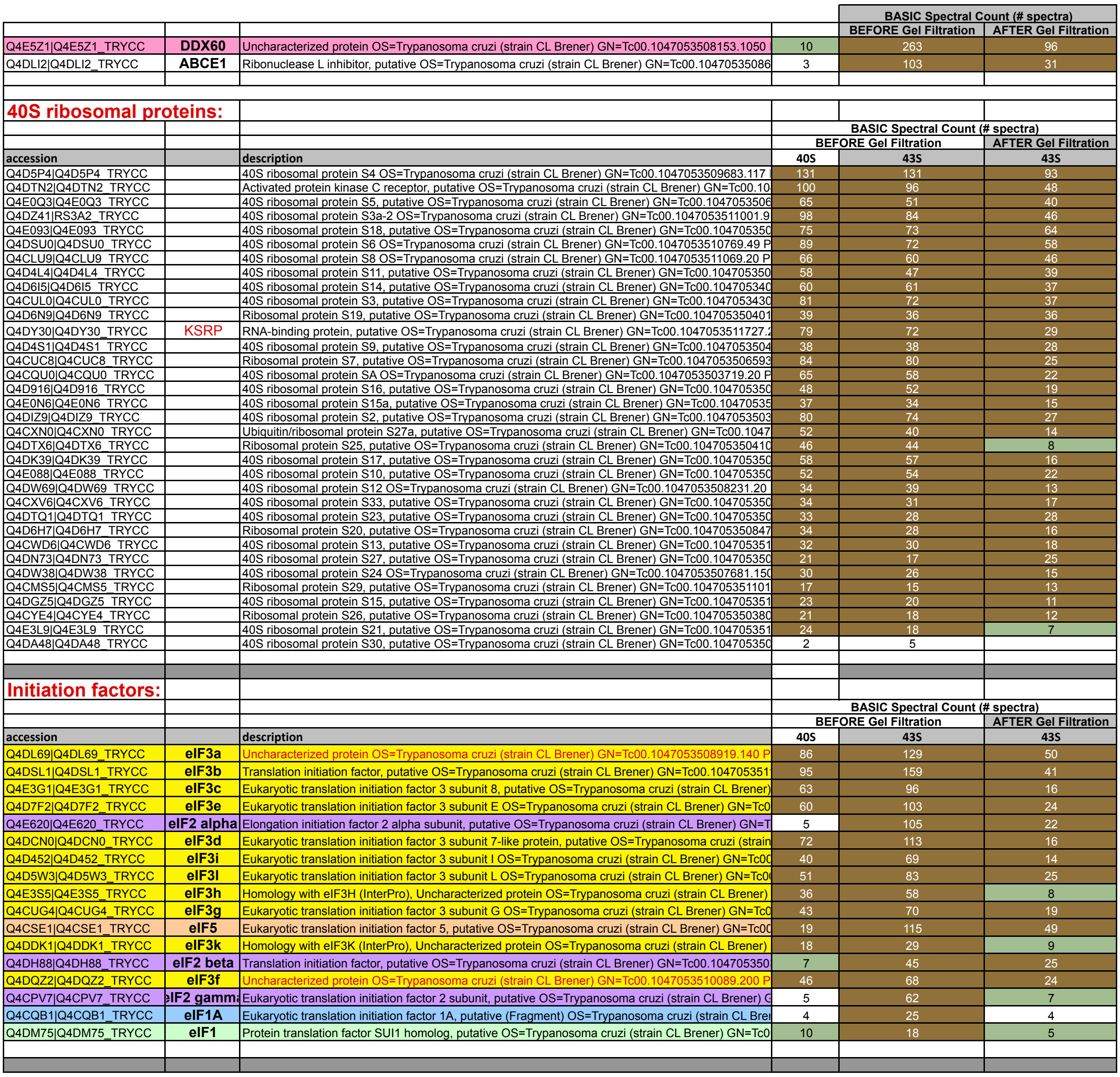
```
Score            Expect  Method                        Identities     Positives       Gaps
54.3 bits(129)   1e-10   Compositional matrix adjust.  74/294(25%)    121/294(41%)    28/294(9%)

t.c. k-DDX60 146  SEMFFIDGDSLFMAALSPQSVDWDLIQPLHVIYNAQKLLYDMHCRGARFHVVFFDSLLWI  205
                  S  F IDGDSL+   L  +S  W   Q LH  Y  +  L D+   G +F +VFF     +
h.s. DDX60   36   SNFFVIDGDSLLVTCLGVKSFKWG--QNLHFFYLVECYLVDLLSNGGQFTIVFFKDAEYA  93

t.c. k-DDX60 206  WESAPAKLFMRENLRKTLMSLSENDAKVGLSISNFSSYYSEAFETYVKQWEPEFILMSDG  265
                  +   P  L +R  L   L   +  D +     + FS   S+ ++ +++Q  P F+++S+
h.s. DDX60   94   YFDFPELLSLRTALILHLQHNTNIDVQ-----TEFSGCLSQDWKLFLEQHYPYFLIVSE-  147

t.c. k-DDX60 266  EQLGRLNP-LQAFFVRPSAANNTHDSRRKANYTDADEANPYRLRRQYRSIV---EDEAVG  321
                  E L  L   L  F+   S     +      + +D      Y +    R+     E+E V
h.s. DDX60   148  EGLSDLQTYLFNFLIIHSWGMKVNVVLSSGHESDTLRFYAYTMESTDRNQTFSKENETVI  207

t.c. k-DDX60 322  DKAALYYRCIHLWAATRRLKVAYSSRIIYKENAM-----VVFTVR---VDGASFERAVTI  373
                    A  Y   I      R L +A     +   + M      +F ++    +G+  +R + +
h.s. DDX60   208  QSA--YKSLIQHLEEIRVLVLATHFEHLKWNDMMEEAYQTLFLLQHLWSEGSDIQRVLCV  265

t.c. k-DDX60 374  ESDVQALAQSMEHEVQLPAISSASLGLLDEEDLSCRERVVYAALHAYLRASARS  427
                   S   +L+  M H V    + S  L L + ED  CR R +  A   +L  S R+
h.s. DDX60   266  TS--CSLSLRMYHRV---LVHSNCLSLQEVEDF-CRLRCLCVAFQLHLPLSQRA  313
```

```
Score            Expect  Method                        Identities     Positives       Gaps
116 bits(290)    2e-29   Compositional matrix adjust.  90/278(32%)    128/278(46%)    31/278(11%)

t.c. k-DDX60 1482 VLRELIARDMGPTIVFSFESEDCGDLVKYVVEQLEEAESRYRKTNEPALYKARIERAAAA  1541
                  ++ +L   D  P I F F+++D G     V   LE+ E++     E   Y        A
h.s. DDX60   1097 LVEKLRQMDKLPAIFFLFKNDDVGKRAGSVCTFLEKTETKSHPHTECHSY------VFAI  1150

t.c. k-DDX60 1542 QEARRKQRESTLKQKRLTTGDDGDVEVADRDMSDGEGEDELFVVPDVLPEFTFIGEKCTV  1601
                   E   K R++   QKR+T  +    E  +R                +L     I E CT
h.s. DDX60   1151 DEVLEKVRKT---QKRITKKNPKKAEKLERKKVYRAEYINFLENLKILE----ISEDCTY  1203

t.c. k-DDX60 1602 EPEVVDSLMEDCEKEGEDLLLRAL----------------QRGIGMHHAGVKGKLRAHVE  1645
                      V +L  +  +  +  L R L                QRGIG HH+ +  K +  VE
h.s. DDX60   1204 AD--VKALHTEITRNKDSTLDRVLPRVRFTRHGKELKALAQRGIGYHHSSMYFKEKEFVE  1261

t.c. k-DDX60 1646 RLFRGRHCGVIFSTETLALGIHSPCRSVVLAGDHILLNPTQFRQMMGRAGRRGLDYLGHL  1705
                   LF      V+ +TETLALGIH PC+SVV A D + L+   +RQM GRAGRRG D LG++
h.s. DDX60   1262 ILFVKGLIRVVTATETLALGIHMPCKSVVFAQDSVYLDALNYRQMSGRAGRRGQDLLGNV  1321

t.c. k-DDX60 1706 VFLGITMRRIKRLMTSSMTVIKGNVQMDPISNLRLLQL  1743
                   F  I + +IKRL+ SS+  ++G   +     LRL+ L
h.s. DDX60   1322 YFFDIPLPKIKRLLASSVPELRGQFPLSITLVLRLMLL  1359
```

```
Score            Expect  Method                        Identities     Positives       Gaps
220 bits(561)    3e-61   Compositional matrix adjust.  143/409(35%)   206/409(50%)    60/409(14%)

t.c. k-DDX60 980  GTLLPSLLPQDKQ-----LFETPEMLQLIHMGHLLERPFVREHDYRVA-FNPDNWQRELL  1033
                   +L P+L+  DK      +   P   QL +MGH L R   ++ D RV  F P+ WQ+ELL
h.s. DDX60   693  NSLDPTLIGDDKNKKKYSIDIGPARFQLQYMGHYLIRDERKDRDPRVQDFIPNAWQQELL  752

t.c. k-DDX60 1034 DIVDGRGSAVVCAPTSAGKTFISYYCMYKALRRTNKKVVVYLAPARALINQAVADVCARY  1093
                  D+VD   SAV+ APTS+GKT+ SYYCM K LR ++  VVVY+APA++L+ Q  A V  R+
h.s. DDX60   753  DVVDKNESAVIVAPTSSGKTYASYYCMEKVLRESDVGVVVYVAPAKSLVGQVAATVENRF  812

t.c. k-DDX60 1094 GSKKYKNPGRYIYGALGGADYHQPHDSCQVLLTVPETFETMLLSPKYTDWVELIDYVILD  1153
                     K    GR + GA    DY     +CQVL+TVPE FE +LL+P    WVE I YVI D
h.s. DDX60   813  T--KTLPAGRTLCGAF-TRDYRHNVLNCQVLITVPECFEILLLAPHRQKWVERIRYVIFD  869

t.c. k-DDX60 1154 EIHSMESNGNGDVWERILALLPCPFVALSATLGETQQLCSWLNRVQGRLKEQTEEM----  1209
                  E+H +        WE +L ++ CPF+ LSAT+     L  WL  V+   K+  + M
h.s. DDX60   870  EVHYLGREVGAKFWELLLVIIRCPFLVLSATINNPNLLTKWLQSVKQYWKQADKIMEEKC  929

t.c. k-DDX60 1210 ----------------SGKMRDFEVHLLPSEGKSIQRWNDIKKYIYLPPPGAALTQKKIK  1253
                                  S K + +EV L+       +R+ND++K+I
h.s. DDX60   930  ISEKQADKCLNFLQDHSYKNQSYEVRLVLCG----ERYNDLEKHI-------------CS  972

t.c. k-DDX60 1254 AQYNNCYIRDLHPLSILTADQLQR-GFPPDISLVPSEVVSLFEKMHSKFNEVVWPNYSSL  1312
                   ++++ Y    HP +  LT D +++ GFPPD++L P E + L++ M   +    WP
h.s. DDX60   973  VKHDDVYFDHPHPCAALTTDIIEKYGFPPDLTLTPQESIQLYDTMAQVWE--TWP-----  1025

t.c. k-DDX60 1313 QLAKTLRAQLMLIEPSKYFEAETYITQERARQYEAEVKNAFAYWAYLGH  1361
                        RAQ +  E    F+ +  I +  AR+YE  +K     W   G
h.s. DDX60   1026 ------RAQELCPEEFILFKNKIVIKKLDARKYEENLKAELTNWIKNGQ  1068
```

Supplementary Figure 8. *T. cruzi* 18S k-DDX60 conservation and secondary structure diagram, Related to Figures 4 and 5. (**A**) BlastP alignment between *T. cruzi* k-DDX60 and human DDX60 showing the relatively modest global homology between both proteins. Only most homologous regions were presented (in green, purple and red boxes). Magenta boxes on domains annotation schema highlight the trypanosomatid-specific domains that are inexistent in DDX60 from human and other eukaryotic species. Pink and violet colors highlight the A-site Insert (AI) and the ATP binding pocket in k-DDX60, respectively. (**B**) Secondary structure elements diagram for k-DDX60 based on its 3D model. Some parts could not be modeled.

SUPPLEMENTARY TABLES



				BASIC Spectral Count (# spectra)	
				BEFORE Gel Filtration	AFTER Gel Filtration
Q4E5Z1|Q4E5Z1_TRYCC	**DDX60**	Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053508153.1050	10	263	96
Q4DLI2|Q4DLI2_TRYCC	**ABCE1**	Ribonuclease L inhibitor, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.10470535086	3	103	31

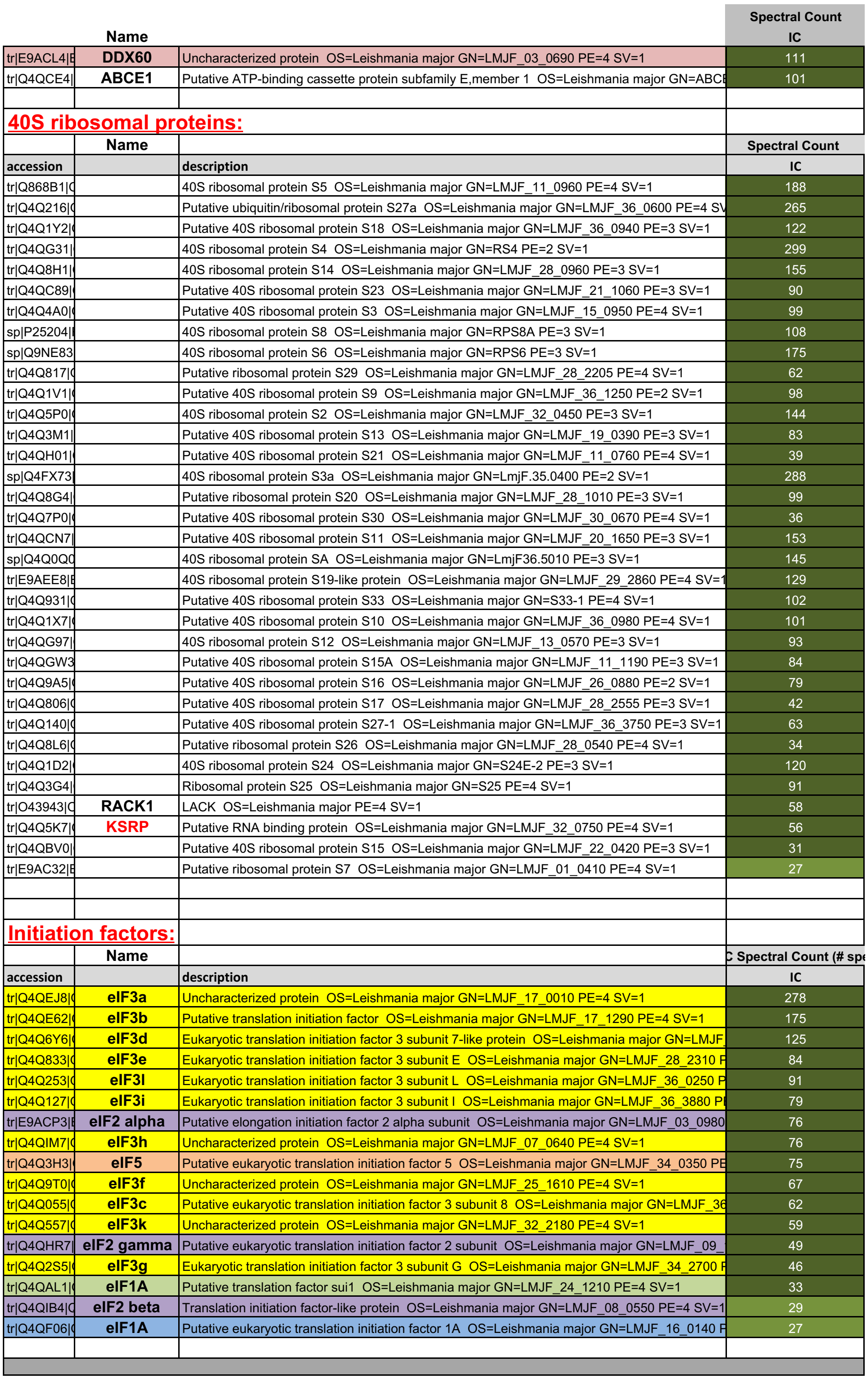
40S ribosomal proteins:

			BASIC Spectral Count (# spectra)		
			BEFORE Gel Filtration		AFTER Gel Filtration
accession		**description**	**40S**	**43S**	**43S**
Q4D5P4|Q4D5P4_TRYCC		40S ribosomal protein S4 OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053509683.117	131	131	93
Q4DTN2|Q4DTN2_TRYCC		Activated protein kinase C receptor, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.10	100	96	48
Q4E0Q3|Q4E0Q3_TRYCC		40S ribosomal protein S5, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053506	65	51	40
Q4DZ41|RS3A2_TRYCC		40S ribosomal protein S3a-2 OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053511001.9	98	84	46
Q4E093|Q4E093_TRYCC		40S ribosomal protein S18, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	75	73	64
Q4DSU0|Q4DSU0_TRYCC		40S ribosomal protein S6 OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053510769.49 P	89	72	58
Q4CLU9|Q4CLU9_TRYCC		40S ribosomal protein S8 OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053511069.20 P	66	60	46
Q4D4L4|Q4D4L4_TRYCC		40S ribosomal protein S11, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	58	47	39
Q4D6I5|Q4D6I5_TRYCC		40S ribosomal protein S14, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705340	60	61	37
Q4CUL0|Q4CUL0_TRYCC		40S ribosomal protein S3, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053430	81	72	37
Q4D6N9|Q4D6N9_TRYCC		Ribosomal protein S19, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350401	39	36	36
Q4DY30|Q4DY30_TRYCC	KSRP	RNA-binding protein, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053511727.	79	72	29
Q4D4S1|Q4D4S1_TRYCC		40S ribosomal protein S9, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053504	38	38	28
Q4CUC8|Q4CUC8_TRYCC		Ribosomal protein S7, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053506593	84	80	25
Q4CQU0|Q4CQU0_TRYCC		40S ribosomal protein SA OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053503719.20 P	65	58	22
Q4D916|Q4D916_TRYCC		40S ribosomal protein S16, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	48	52	19
Q4E0N6|Q4E0N6_TRYCC		40S ribosomal protein S15a, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.10470535	37	34	15
Q4DIZ9|Q4DIZ9_TRYCC		40S ribosomal protein S2, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053503	80	74	27
Q4CXN0|Q4CXN0_TRYCC		Ubiquitin/ribosomal protein S27a, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047	52	40	14
Q4DTX6|Q4DTX6_TRYCC		Ribosomal protein S25, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350410	46	44	8
Q4DK39|Q4DK39_TRYCC		40S ribosomal protein S17, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	58	57	16
Q4E088|Q4E088_TRYCC		40S ribosomal protein S10, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	52	54	22
Q4DW69|Q4DW69_TRYCC		40S ribosomal protein S12 OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053508231.20	34	39	13
Q4CXV6|Q4CXV6_TRYCC		40S ribosomal protein S33, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	34	31	17
Q4DTQ1|Q4DTQ1_TRYCC		40S ribosomal protein S23, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	33	28	28
Q4D6H7|Q4D6H7_TRYCC		Ribosomal protein S20, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350847	34	28	16
Q4CWD6|Q4CWD6_TRYCC		40S ribosomal protein S13, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705351	32	30	18
Q4DN73|Q4DN73_TRYCC		40S ribosomal protein S27, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	21	17	25
Q4DW38|Q4DW38_TRYCC		40S ribosomal protein S24 OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053507681.15	30	26	15
Q4CMS5|Q4CMS5_TRYCC		Ribosomal protein S29, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705351101	17	15	13
Q4DGZ5|Q4DGZ5_TRYCC		40S ribosomal protein S15, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705351	23	20	11
Q4CYE4|Q4CYE4_TRYCC		Ribosomal protein S26, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350380	21	18	12
Q4E3L9|Q4E3L9_TRYCC		40S ribosomal protein S21, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705351	24	18	7
Q4DA48|Q4DA48_TRYCC		40S ribosomal protein S30, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	2	5	

Initiation factors:

			BASIC Spectral Count (# spectra)		
			BEFORE Gel Filtration		AFTER Gel Filtration
accession		**description**	**40S**	**43S**	**43S**
Q4DL69|Q4DL69_TRYCC	**eIF3a**	Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053508919.140 P	86	129	50
Q4DSL1|Q4DSL1_TRYCC	**eIF3b**	Translation initiation factor, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705351	95	159	41
Q4E3G1|Q4E3G1_TRYCC	**eIF3c**	Eukaryotic translation initiation factor 3 subunit 8, putative OS=Trypanosoma cruzi (strain CL Brener)	63	96	16
Q4D7F2|Q4D7F2_TRYCC	**eIF3e**	Eukaryotic translation initiation factor 3 subunit E OS=Trypanosoma cruzi (strain CL Brener) GN=Tc0	60	103	24
Q4E620|Q4E620_TRYCC	**eIF2 alpha**	Elongation initiation factor 2 alpha subunit, putative OS=Trypanosoma cruzi (strain CL Brener) GN=T	5	105	22
Q4DCN0|Q4DCN0_TRYCC	**eIF3d**	Eukaryotic translation initiation factor 3 subunit 7-like protein, putative OS=Trypanosoma cruzi (strain	72	113	16
Q4D452|Q4D452_TRYCC	**eIF3i**	Eukaryotic translation initiation factor 3 subunit I OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00	40	69	14
Q4D5W3|Q4D5W3_TRYCC	**eIF3l**	Eukaryotic translation initiation factor 3 subunit L OS=Trypanosoma cruzi (strain CL Brener) GN=Tc0	51	83	25
Q4E3S5|Q4E3S5_TRYCC	**eIF3h**	Homology with eIF3H (InterPro), Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener)	36	58	8
Q4CUG4|Q4CUG4_TRYCC	**eIF3g**	Eukaryotic translation initiation factor 3 subunit G OS=Trypanosoma cruzi (strain CL Brener) GN=Tc0	43	70	19
Q4CSE1|Q4CSE1_TRYCC	**eIF5**	Eukaryotic translation initiation factor 5, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00	19	115	49
Q4DDK1|Q4DDK1_TRYCC	**eIF3k**	Homology with eIF3K (InterPro), Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener)	18	29	9
Q4DH88|Q4DH88_TRYCC	**eIF2 beta**	Translation initiation factor, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.104705350	7	45	25
Q4DQZ2|Q4DQZ2_TRYCC	**eIF3f**	Uncharacterized protein OS=Trypanosoma cruzi (strain CL Brener) GN=Tc00.1047053510089.200 P	46	68	24
Q4CPV7|Q4CPV7_TRYCC	**eIF2 gamm**	Eukaryotic translation initiation factor 2 subunit, putative OS=Trypanosoma cruzi (strain CL Brener) G	5	62	7
Q4CQB1|Q4CQB1_TRYCC	**eIF1A**	Eukaryotic translation initiation factor 1A, putative (Fragment) OS=Trypanosoma cruzi (strain CL Bre	4	25	4
Q4DM75|Q4DM75_TRYCC	**eIF1**	Protein translation factor SUI1 homolog, putative OS=Trypanosoma cruzi (strain CL Brener) GN=Tc0	10	18	5

Supplementary Table 1. Mass-spectrometry analysis of the *T. cruzi* 43S PIC, Related to Figure 1. Composition of the *T. cruzi* 43S PIC in 40S ribosomal proteins and initiation factors. K-DDX60 and ABCE1 were singled out. The analysis compares the 43S related fractions without (labeled 40S) and with GMP-PNP (labeled 43S), before and after Gel-filtration. Accessions, description and spectral counts are indicated for each fraction. Full dataset can be found at the PRIDE partner repository with the dataset identifier PXD016063 (See Methods).



	Name		Spectral Count IC
tr|E9ACL4|	**DDX60**	Uncharacterized protein OS=Leishmania major GN=LMJF_03_0690 PE=4 SV=1	111
tr|Q4QCE4|	**ABCE1**	Putative ATP-binding cassette protein subfamily E,member 1 OS=Leishmania major GN=ABC	101
40S ribosomal proteins:			
	Name		**Spectral Count**
accession		**description**	**IC**
tr|Q868B1|		40S ribosomal protein S5 OS=Leishmania major GN=LMJF_11_0960 PE=4 SV=1	188
tr|Q4Q216|		Putative ubiquitin/ribosomal protein S27a OS=Leishmania major GN=LMJF_36_0600 PE=4 SV	265
tr|Q4Q1Y2|		Putative 40S ribosomal protein S18 OS=Leishmania major GN=LMJF_36_0940 PE=3 SV=1	122
tr|Q4QG31|		40S ribosomal protein S4 OS=Leishmania major GN=RS4 PE=2 SV=1	299
tr|Q4Q8H1|		40S ribosomal protein S14 OS=Leishmania major GN=LMJF_28_0960 PE=3 SV=1	155
tr|Q4QC89|		Putative 40S ribosomal protein S23 OS=Leishmania major GN=LMJF_21_1060 PE=3 SV=1	90
tr|Q4Q4A0|		Putative 40S ribosomal protein S3 OS=Leishmania major GN=LMJF_15_0950 PE=4 SV=1	99
sp|P25204|		40S ribosomal protein S8 OS=Leishmania major GN=RPS8A PE=3 SV=1	108
sp|Q9NE83		40S ribosomal protein S6 OS=Leishmania major GN=RPS6 PE=3 SV=1	175
tr|Q4Q817|		Putative ribosomal protein S29 OS=Leishmania major GN=LMJF_28_2205 PE=4 SV=1	62
tr|Q4Q1V1|		Putative 40S ribosomal protein S9 OS=Leishmania major GN=LMJF_36_1250 PE=2 SV=1	98
tr|Q4Q5P0|		40S ribosomal protein S2 OS=Leishmania major GN=LMJF_32_0450 PE=3 SV=1	144
tr|Q4Q3M1|		Putative 40S ribosomal protein S13 OS=Leishmania major GN=LMJF_19_0390 PE=3 SV=1	83
tr|Q4QH01|		Putative 40S ribosomal protein S21 OS=Leishmania major GN=LMJF_11_0760 PE=4 SV=1	39
sp|Q4FX73|		40S ribosomal protein S3a OS=Leishmania major GN=LmjF.35.0400 PE=2 SV=1	288
tr|Q4Q8G4|		Putative ribosomal protein S20 OS=Leishmania major GN=LMJF_28_1010 PE=3 SV=1	99
tr|Q4Q7P0|		Putative 40S ribosomal protein S30 OS=Leishmania major GN=LMJF_30_0670 PE=4 SV=1	36
tr|Q4QCN7|		Putative 40S ribosomal protein S11 OS=Leishmania major GN=LMJF_20_1650 PE=3 SV=1	153
sp|Q4Q0Q0		40S ribosomal protein SA OS=Leishmania major GN=LmjF36.5010 PE=3 SV=1	145
tr|E9AEE8|		40S ribosomal protein S19-like protein OS=Leishmania major GN=LMJF_29_2860 PE=4 SV=1	129
tr|Q4Q931|		Putative 40S ribosomal protein S33 OS=Leishmania major GN=S33-1 PE=4 SV=1	102
tr|Q4Q1X7|		Putative 40S ribosomal protein S10 OS=Leishmania major GN=LMJF_36_0980 PE=4 SV=1	101
tr|Q4QG97|		40S ribosomal protein S12 OS=Leishmania major GN=LMJF_13_0570 PE=3 SV=1	93
tr|Q4QGW3		Putative 40S ribosomal protein S15A OS=Leishmania major GN=LMJF_11_1190 PE=3 SV=1	84
tr|Q4Q9A5|		Putative 40S ribosomal protein S16 OS=Leishmania major GN=LMJF_26_0880 PE=2 SV=1	79
tr|Q4Q806|		Putative 40S ribosomal protein S17 OS=Leishmania major GN=LMJF_28_2555 PE=3 SV=1	42
tr|Q4Q140|		Putative 40S ribosomal protein S27-1 OS=Leishmania major GN=LMJF_36_3750 PE=3 SV=1	63
tr|Q4Q8L6|		Putative ribosomal protein S26 OS=Leishmania major GN=LMJF_28_0540 PE=4 SV=1	34
tr|Q4Q1D2|		40S ribosomal protein S24 OS=Leishmania major GN=S24E-2 PE=3 SV=1	120
tr|Q4Q3G4|		Ribosomal protein S25 OS=Leishmania major GN=S25 PE=4 SV=1	91
tr|O43943|	**RACK1**	LACK OS=Leishmania major PE=4 SV=1	58
tr|Q4Q5K7|	**KSRP**	Putative RNA binding protein OS=Leishmania major GN=LMJF_32_0750 PE=4 SV=1	56
tr|Q4QBV0|		Putative 40S ribosomal protein S15 OS=Leishmania major GN=LMJF_22_0420 PE=3 SV=1	31
tr|E9AC32|		Putative ribosomal protein S7 OS=Leishmania major GN=LMJF_01_0410 PE=4 SV=1	27
Initiation factors:			
	Name		**Spectral Count (# spe**
accession		**description**	**IC**
tr|Q4QEJ8|	**eIF3a**	Uncharacterized protein OS=Leishmania major GN=LMJF_17_0010 PE=4 SV=1	278
tr|Q4QE62|	**eIF3b**	Putative translation initiation factor OS=Leishmania major GN=LMJF_17_1290 PE=4 SV=1	175
tr|Q4Q6Y6|	**eIF3d**	Eukaryotic translation initiation factor 3 subunit 7-like protein OS=Leishmania major GN=LMJF	125
tr|Q4Q833|	**eIF3e**	Eukaryotic translation initiation factor 3 subunit E OS=Leishmania major GN=LMJF_28_2310	84
tr|Q4Q253|	**eIF3l**	Eukaryotic translation initiation factor 3 subunit L OS=Leishmania major GN=LMJF_36_0250	91
tr|Q4Q127|	**eIF3i**	Eukaryotic translation initiation factor 3 subunit I OS=Leishmania major GN=LMJF_36_3880	79
tr|E9ACP3|	**eIF2 alpha**	Putative elongation initiation factor 2 alpha subunit OS=Leishmania major GN=LMJF_03_0980	76
tr|Q4QIM7|	**eIF3h**	Uncharacterized protein OS=Leishmania major GN=LMJF_07_0640 PE=4 SV=1	76
tr|Q4Q3H3|	**eIF5**	Putative eukaryotic translation initiation factor 5 OS=Leishmania major GN=LMJF_34_0350 P	75
tr|Q4Q9T0|	**eIF3f**	Uncharacterized protein OS=Leishmania major GN=LMJF_25_1610 PE=4 SV=1	67
tr|Q4Q055|	**eIF3c**	Putative eukaryotic translation initiation factor 3 subunit 8 OS=Leishmania major GN=LMJF_36	62
tr|Q4Q557|	**eIF3k**	Uncharacterized protein OS=Leishmania major GN=LMJF_32_2180 PE=4 SV=1	59
tr|Q4QHR7|	**eIF2 gamma**	Putative eukaryotic translation initiation factor 2 subunit OS=Leishmania major GN=LMJF_09_	49
tr|Q4Q2S5|	**eIF3g**	Eukaryotic translation initiation factor 3 subunit G OS=Leishmania major GN=LMJF_34_2700	46
tr|Q4QAL1|	**eIF1A**	Putative translation factor sui1 OS=Leishmania major GN=LMJF_24_1210 PE=4 SV=1	33
tr|Q4QIB4|	**eIF2 beta**	Translation initiation factor-like protein OS=Leishmania major GN=LMJF_08_0550 PE=4 SV=1	29
tr|Q4QF06|	**eIF1A**	Putative eukaryotic translation initiation factor 1A OS=Leishmania major GN=LMJF_16_0140	27

Supplementary Table 2. Mass-spectrometry analysis of the *L. Tarentolae* 43S PIC, Related to Figure 1. Composition of the *L. Tarentolae* 43S PIC in 40S ribosomal proteins and initiation factors. K-DDX60 and ABCE1 were singled out. The analysis of the 43S related fraction was made after supplementation with GMP-PNP (IC), before Gel-filtration. Accessions, description and spectral counts are indicated. Full dataset can be found at the PRIDE partner repository with the dataset identifier PXD016063 (See Methods).

	Ribosomal RNA	Ribosomal protein	Initiation factors
eIF1	N65-G2303, C64-G2303, Q81-C2282, R33-A1341, R33-G2283, K37-G2283, R56-G2303, R61-C2183	**none**	**eIF2-β : R29-S251, Q31-S327, Q43-T325, H27-T325, V77-Y326** **eIF2-γ : S16-N459, V17-V147, Q12-Q412, L21-V85, Q13-V147** **eIF3c : L49-F36, I54-W35 R53-E37, R52-T39, N96-R26, L49-I31**
eIF1A	N48-A2277, R66-C620, W74-A2279, **R155-G1685**	**eS30 :** E35-R10, F88-L8 **uS13 : D162-R119, L164-V124** **uS19 : V158-V100, V158-A82, V158-A111** **uS12 :** F88-L91	**eIF2-β : Y133-L282, V134-N208, F135-P213, F135-Y279**
eIF2-α	**none**	**uS7 :**, Y200-K177, Y200-D180, Y200-R184, T148-R122, Y166-V120, T167-R122, D195-R184	**tRNA :** K104-C55, R105-G52, R108-U54, W119-C55, H 232-C55, E296-U54 **eIF2-γ :** R331-E279, F315-L321, V320-L350, P350-F268
eIF2-β	**R333-U1340, R333-G1342, R337-U1339, R337-U1340**	**uS19 :** N259-R137	**tRNA :** K221-A36, N255-G25, K300-G68, R303-G69, **eIF1 :** S251-R29, E267-Q32, T325-Q43, Y326-V77, Y326-H27, S323-R29 **eIF1A : N208-V134, P213-F135, Y279-F135, L282-Y133** **eIF5 : N118-R265, L120-V329, L120-A262, L123-V325, K125-Q364, V132-W372, L142-F331** **eIF2-γ :** N173-H248, T176-Y245, G181-Y241, Y182-Y211, Y184-D240, S185-N238, R189-E204, M305-E83, T317-M86, T317-E83
eIF2-γ	**none**	**none**	**tRNA :** K79-C73, D269-A75, K272-A72, R282-A75 **eIF1 : V85-L21, I88-L21, V147-V17, N459-S16, Q412-Q12** **eIF2-α :** E279-R331, L321- F315, L350- V320, F268- P350 **eIF2-β :** H248- N173, Y245- T176, Y241- G181, Y211- Y182, D240- Y184, N238- S185, E204- R189, E83- M305, M86- T317, E83-T317 **eIF5 : S224-R230, D219-R229, S220-R273, F383-L240, N430-T205, P431-D204, W465-T237, R469-T205** **k-DDX60: P171-P770, D209-R902**
eIF3c	**S52-A1360, R53-C1361, K56-C1596, R127-C370, Q204-U1526, K207-A1525 R215-A1523, R232-U1476 and U1478, Q329-G1438, R331-U1439, R243-U1526**	**eS27 :** Q191-Q56, K192-E54	**eIF1 : I31-M97, I31-L49, F36-L49, E37-R53, W35-F91, W35-I54, T39-R52** **eIF3d : P234-W44, R295-W44, L489-W44, L233-A47, L380-F9, L418-W16, R419-P13, I434-M28, N437-D26** **k-DDX60: N-ter tail with Y832 and F834**
eIF3a		**eS1 :** T7-Q77, R8-T77, T12-R192, L17-I194	
eIF3d	**D43-G1532, D50-A1475, R149-U1863 and U1862, R294-U1866 and C1867, D306-U1864,Q296-G1861, K301-U1863**	**eS27 : T36-K37, I39-F80, I39-L74** **S33 :** R219-E76, D255-R83, K371-M98, Q368-K94, L435-M73 **uS7 :** Q434-E21, Q368-D26, E368-R51 **RACK1 :** S409-E277, N410-Q279	**eIF3c : F9-L380, P13-R419, W16-L418, D26-N437, W44-P234, W44-R295, W44-L489, A47-L233, M28-I434** **eIF3e : F3-T198, L5-A196, P6-T198, W16-I246, W16-Q247, E7-T245 ; P13-T248**
eIF5	**none**	**none**	**eIF2-β : A262-L120, R265-N118, V325-L123, V329-L120, I332-L142, Q364-K125, W372-V132** **eIF2-γ : D204-P431, T205-R469, T205-N430, R229-D219, R230- S224, T237-W465, L240-F383, R273-S220** **k-DDX60: D284-S944, D288-R941, K292-S826**
k-DDX60	**S26-U1722, R95-U1723, K724-A51, Q725-A51, H728-G477**	**eS12 : S3-D70, R6-E72** **eS31 : Y5-K94, E92-L92, E93-K94** **uS12 : R739-Q73, D744-N97 T740-N97**	**tRNA : Q1548-A34, S1551-A34** **eIF2-γ : P770-P171, R902-D209** **eIF3c : Y832 and F834 with N-ter tail** **eIF5 : S826-K292, R941-D288, S944-D284**

Supplementary Table 3. Detailed overview of interactions between eIFs, ribosomal proteins, rRNA and k-DDX60, Related to Figures 2, 3 and 5. Novel interactions revealed by analysis are colored in deep blue. Most of these novel interactions are shown in ribbons and sticks models fitted into their corresponding densities in Fig. S 3 and 4.

Data Collection	***T. cruzi* 43S**	***T. cruzi* 43S + ATP**	***L. tarentolae* 43S**
Microscope	Titan Krios	Titan Krios	Talos Artica
Voltage (kV)	300	300	200
Magnification	127,272	127,272	120,000
Pixel size (Å)	1.1	1.1	1.21
Detector	Gatan Summit K2	Gatan Summit K2	Falcon III
Defocus range (µm)	-0.6 to -4.5	-0.6 to -4.5	-0.6 to -3.0
Tot. electron exposure ($e^{-}\text{Å}^{-2}$)	30	30	40
Exposure rate ($e^{-}\text{Å}^{-2}\text{frame}^{-1}$)	1.5	1.5	2.0
Data collection software	SerialEM	EPU	EPU
Data Processing			
Independent data collections	1	1	1
Useable micrographs	3271	2638	?
Particles	202920	98840	52302
Final particles (43S)	33775	19700	10144
Accuracy translations (pix) / rotations (°)	0.432/0.234375°	0.432/0.234375°	0.432/0.234375°
Resolution (Å, 0.143 FSC)	3.33	4.3	8.1
Local resolution range (Å)	2.5-6	N/A	N/A
Model Composition			
Chains	59	N/A	N/A
Non-hydrogen atoms	136893	N/A	N/A
Protein residues	11196	N/A	N/A
RNA bases	2225	N/A	N/A
Refinement			
Software	Phenix_ValidationEM	N/A	N/A
Resolution (Å)	3.33	N/A	N/A
CC (mask)	0.57	N/A	N/A
CC (main chain)	0.5	N/A	N/A
CC (side chain)	0.58	N/A	N/A
R.M.S deviations			
Bond lengths (Å)	0.020	N/A	N/A
Bond angles (°)	2.209	N/A	N/A
Validation			
Molprobity score	2.65	N/A	N/A
Clashscore, all atoms	9.20	N/A	N/A
Rotamers outliers (%)	3.48	N/A	N/A
Cβ outliers (%)	1.41	N/A	N/A
CaBLAM outliers (%)	10.9	N/A	N/A
Ramachandran plot			
Favored (%)	79.09	N/A	N/A
Allowed (%)	14.19	N/A	N/A
Outliers (%)	6.72	N/A	N/A

Supplementary Table 4. Data collection, processing, refinement and model statistics, Related to Figure 1 and STAR Methods section. A near complete atomic model was only derived for the highest resolution cryo-EM reconstruction, i.e. the *T. cruzi* 43S PIC stalled with GMP-PNP. The *T. cruzi* 43S PIC stalled with GMP-PNP supplemented with ATP presents a lower resolution and therefore we didn't derive a full atomic model, instead we flexibly fitted k-DDX60 only into its map to illustrate its conformational changes.

Primer	Sequence
AH-h3c-PmeI	ATATAGTTTAAACGCCATGTCGCGGTTTTTCACC
AH-h3c-FseI	ATATAGGCCGGCCTCAGTAGGCCGTCTGAGACTG
AH-h3a-PmeI	ATATAGTTTAAACAAGATGCCGGCCTATTTTCAG
AH-h3a-FseI	ATATAGGCCGGCCTTAACGTCGTACTGTGGTCCA
AH-h3m-EcoRI	ATATAGAATTCACCATGAGCGTCCCGGCCTTC
AH-h3m-FseI	ATATAGGCCGGCCTCAGGTATCAGAAAGACTCAA
AH-h3k-EcoRI	ATATAGAATTCGTCATGGCGATGTTTGAGCAG
AH-h3k-FseI	ATATAGGCCGGCCTTACTGGGAGGAGGCCATGAT
AH-h3d-EcoRI	ATATAGAATTCAAGATGGCAAAGTTCATGACA
AH-h3d-FseI	ATATAGGCCGGCCTTAAGTTTCTTCCTCTTCTTCTTCCTC
AH-h3e-EcoRI	ATATAGAATTCAAGATGGCGGAGTACGACTTG
AH-h3e-FseI	ATATAGGCCGGCCTCAGTAGAAGCCAGAATCTTG
DS-eIF1-BamHI	ATCGGATCCATATGTCCGCTATCCAGAACC
DS-eIF1-SalI	TGTGTCGACTTAAAACCCATGAACCTTCAG
SW-heIF5-EcoRI	ATAGAATTCGATGTCTGTCAATGTCAACC
SW-heIF5-SalI-R	ACTAGTCGACTTAAATGGCATCAATATCG
DS-eIF2β-BamHI	ATCGGATCCATATGTCTGGGGACGAGATG
DS-eIF2β-SalI	CGTGTCGACTTAGTTAGCTTTGGCACG
AH-h3c-325-FseI	ATATACGGCCGGCCTCAGGTGATCTCAGTTCCCTTGGC
TS-h3c-326-PmeI	ATATAGTTTAAACGCCATGCATGCTGTTGTTATCAAGAAACTG
TS-h3c-30-325-PmeI	ATATAGTTTAAACGCCATGAACTATGGCAAACAGCCATTG
TP-h3c-130-325-PmeI	GCCGCGTTTAAACGCCATGAACAAGAACAATGCCAAGGC
TP-pGL4-CMV-heIF2β-EcoRI	CGCCAGAATTCACCATGTCTGGGGACGAGATGATT
TP-pGL4-CMV-eIF2β-FseI	ATATAGGCCGGCCTTAGTTAGCTTTGGCACGGAG
TP-pGL4-CMV-eIF2β-1-309-FseI	CGCCAGGCCGGCCTTAACATCTAGAATGACAAGTTTC
TP-pGEX-5X3-teIF1-BamHI	ACCTAGGATCCCCATGCTAAACAACGAGCTCGCTAACC
TP-pGEX-5X3-teIF1-SalI	ACCGAGTCGACTTAGTTCAGAGAGTGGATCTC
TP-pGEX-5X3-teIF5-BamHI	ACCGAGGATCCCCATGTCGGTTCCAATGATACCCATTG
TP-pGEX-5X3-teIF5-SalI	CGCTAGTCGACCTATGTCGATCCTACAAGCCATTCGAC
TP-pGL4-teIF5-EcoRI	GCTGTGAATTCGCCACCATGTCGGTTCCAATGATACCC
TP-pGL4-teIF5-FseI	TATATGGCCGGCCCTATGTCGATCCTACAAGCCATTC
TP-pGL4-CMV-teIF3c-PmeI	CGCCGGTTTAAACACCATGAGCAACTTTTTTGATGTCAGCGACAGTG
TP-pGL4-CMV-teIF3c-FseI	ATATAGGCCGGCCGTTAAAATCCTCCTCTACCACGTCCTCGAC
TP-pGL4-CMV-teIF3c-14-PmeI	CGCGCGTTTAAACACCATGCTGGATGAGGTCATACATCACGATG
TP-pGL4-CMV-teIF3c-39-PmeI	AGCCAGTTTAAACACCATGACCGATGATGAGGACGCGGATG
TP-pGL4-CMV-teIF3c-172-FseI	TATATGGCCGGCCGTTACTCCTCACCCTGTCCTTCATC
TP-pGEX-teIF3c-BamHI	GCGGCGGATCCCCATGAGCAACTTTTTTGATGTC
TP-pGEX-teIF3c-1-172-EcoRI	GCCGCGAATTCCTTTACTCCTCACCCTGTCCTTC
TP-pGEX-5X3-eIF3e-BamHI	ATATAGGATCCCCATGGCGGAGTACGACTTGAC
TP-pGEX-5X3-eIF3e-SalI	ATGCCGTCGACTCAGTAGAAGCCAGAATCTTG

TP-pGL4-CMV-h3d-19-EcoRI	CTGCAGAATTCAAGATGTGTGCGGTTCCCGAGCAG
AH-h3d-FseI	ATATAGGCCGGCCTTAAGTTTCTTCCTCTTCTTCTTCCTC
AH-h3d-EcoRI	ATATAGAATTCAAGATGGCAAAGTTCATGACA
TP-pGL4-CMV-h3d-114-FseI	ACGTAGGCCGGCCTTACATGTTCCGACGATCTTTGTC
TP-pGEX-heIF3d-BamHI	ATATCGGATCCCCATGGCAAAGTTCATGACACCC
TP-pGEX-heIF3d-SalI	GCGCGGTCGACTTAAGTTTCTTCCTCTTCTTCTTCCTC
TP-pGEX-eIF3c-EcoRI	ACCGAGAATTCCATGTCGCGGTTTTTCACCACC
TP-pGEX-eIF3c-SalI	TATATGTCGACTCAGTAGGCCGTCTGAGACTG
TP-pGEX-eIF3a-SalI	ACCTAGTCGACATGCCGGCCTATTTTCAGAGG
TP-pGEX-eIF3a-NotI	ATATAGCGGCCGCTTAACGTCGTACTGTGGTCCA
TP-teIF3c-AsiSI	GCACCGCGATCGCATGAGCAACTTTTTTGATGTCAGCG
TP-teIF3c-1-172-MluI	ATATAACGCGTCTCCTCACCCTGTCCTTCATC
TP-teIF5-AsiSI	ACCGAGCGATCGCATGTCGGTTCCAATGATACCC
TP-teIF5-MluI	TCGATACGCGTTGTCGATCCTACAAGCCATTC

Supplementary Table 5. List of primers, Related to STAR Methods section.

Gene string	Sequence 5´-3´
heIF1-box-Ala-102-113	GGCGACCATCCTCCAAAATCGGATCTGATCGAAGGTCGTGGGATCCATATGTCCGCTATCCAGAACCTCCACTCTTTCGACCCCTTTGCTGATGCAAGTAAGGGTGATGACCTGCTTCCTGCTGGCACTGAGGATTATATCCATATAAGAATTCAACAGAGAAACGGCAGGAAGACCCTTACTACTGTCCAAGGGATCGCTGATGATTACGATAAAAAGAAACTAGTGAAGGCGTTTAAGAAAAAGTTTGCCTGCAATGGTACTGTAATTGAGCATCCGGAATATGGAGAAGTAATTCAGCTACAGGGTGACCAACGCAAGAACATATGCCAGTTCCTCGTAGAGATTGGAGCAGCAGCAGCTGCAGCAGCCGCAGCCGCGGCAGCATAAGTCGACTCGAGCGGCCGCATCGTGACTGACTGACGATCTGCCTCGC
pGEX-heIF3e-delta-244-252	GACCATCCTCCAAAATCGGATCTGATCGAAGGTCGTGGGATCCCCATGGCGGAGTACGACTTGACTACTCGCATCGCGCACTTTTTGGATCGGCATCTAGTCTTTCCGCTTCTTGAATTTCTCTCTGTAAAGGAGATATATAATGAAAAGGAATTATTACAAGGTAAATTGGACCTTCTTAGTGATACCAACATGGTAGACTTTGCTATGGATGTATACAAAAACCTTTATTCTGATGATATTCCTCATGCTTTGAGAGAGAAAAGAACCACAGTGGTTGCACAACTGAAACAGCTTCAGGCAGAAACAGAACCAATTGTGAAGATGTTTGAAGATCCAGAAACTACAAGGCAAATGCAGTCAACCAGGGATGGTAGGATGCTCTTTGACTACCTGGCGGACAAGCATGGTTTTAGGCAGGAATATTTAGATACACTCTACAGATATGCAAAATTCCAGTACGAATGTGGGAATTACTCAGGAGCAGCAGAATATCTTTATTTTTTAGAGTGCTGGTTCCAGCAACAGATAGAAATGCTTTAAGTTCACTCTGGGGAAAGCTGGCCTCTGAAATCTTAATGCAGAATTGGGATGCAGCCATGGAAGACCTTACACGGTTAAAAGAGACCATAGATAATAATTCTGTGAGTTCTCCACTTCAGTCTCTTCAGCAGAGAACATGGCTCATTCACTGGTCTCTGTTTGTTTTCTTCAATCACCCCAAAGGTCGCGATAATATTATTGACCTCTTCCTTTATCAGCCACAATATCTTATTCTTCGCTATTTGACTACAGCAGTCATAACAAACAAGGATGTTCGAAAACGTCGGCAGGTTCTAAAAGATCTAGTTAAAGTTATTCAACAGGAGTCTTACACATATAAA
pGEX-heIF3e-I246A-Q247A-T248A	GACCATCCTCCAAAATCGGATCTGATCGAAGGTCGTGGGATCCCCATGGCGGAGTACGACTTGACTACTCGCATCGCGCACTTTTTGGATCGGCATCTAGTCTTTCCGCTTCTTGAATTTCTCTCTGTAAAGGAGATATATAATGAAAAGGAATTATTACAAGGTAAATTGGACCTTCTTAGTGATACCAACATGGTAGACTTTGCTATGGATGTATACAAAAACCTTTATTCTGATGATATTCCTCATGCTTTGAGAGAGAAAAGAACCACAGTGGTTGCACAACTGAAACAGCTTCAGGCAGAAACAGAACCAATTGTGAAGATGTTTGAAGATCCAGAAACTACAAGGCAAATGCAGTCAACCAGGGATGGTAGGATGCTCTTTGACTACCTGGCGGACAAGCATGGTTTTAGGCAGGAATATTTAGATACACTCTACAGATATGCAAAATTCCAGTACGAATGTGGGAATTACTCAGGAGCAGCAGAATATCTTTATTTTTTAGAGTGCTGGTTCCAGCAACAGATAGAAATGCTTTAAGTTCACTCTGGGGAAAGCTGGCCTCTGAAATCTTAATGCAGAATTGGGATGCAGCCATGGAAGACCTTACACGGTTAAAAGAGACCATAGATAATAATTCTGTGAGTTCTCCACTTCAGTCTCTTCAGCAGAGAACATGGCTCATTCACTGGTCTCTGTTTGTTTTCTTCAATCACCCCAAAGGTCGCGATAATATTATTGACCTCTTCCTTTATCAGCCACAATATCTTAATGCAGCTGCGGCAATGTGTCCACACATTCTTCGCTATTTGACTACAGCAGTCATAACAAACAAGGATGTTCGAAAACGTCGGCAGGTTCTAAAAGATCTAGTTAAAGTTATTCAACAGGAGTCTTACACATATAAA

pGL4-CMV-h3c-1-325-d171-240	TGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACGCCATGTCGCGGTTTTTCACCACCGGTTCGGACAGCGAGTCCGAGTCGTCCTTGTCCGGGGAGGAGCTCGTCACCAAACCTGTCGGAGGCAACTATGGCAAACAGCCATTGTTGCTGAGCGAGGATGAAGAAGATACCAAGAGAGTTGTCCGCAGTGCCAAGGACAAGAGGTTTGAGGAGCTGACCAACCTTATCCGGACCATCCGTAATGCCATGAAGATTCGTGATGTCACCAAGTGCCTGGAAGAGTTTGAGCTCCTGGGAAAAGCATATGGGAAGGCCAAAAGCATTGTGGACAAAGAAGGTGTCCCCCGGTTCTATATCCGCATCCTGGCTGACCTAGAGGACTATCTTAATGAGCTTTGGGAAGATAAGGAAGGGAAGAAGAAGATGAACAAGAACAATGCCAAGGCTCTGAGCACCTTGCGTCAGAAGATCCGAAAATACAACCGTGATTTCGAGTCCCATATCACAAGCTACAAGCAGAACCCCGAGCAGTCTGCGGATGAAGATGACTCAGAGGAGGAAGAAGGGAAACAAACCGCGCTGGCCTCAAGATTTCTTAAAAAGGCACCCACCACAGATGAGGACAAGAAGGCAGCCGAGAAGAAACGGGAGGACAAAGCTAAGAAGAAGCACGACAGGAAATCCAAGCGCCTGGATGAGGAGGAGGAGGACAATGAAGGCGGGGAGTGGGAAAGGGTCCGGGGCGGAGTGCCGTTGGTTAAGGAGAAGCCAAAAATGTTTGCCAAGGGAACTGAGATCACCTGAGGCCGGCCGCTTCGAGCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTA
pGL4-CMV-h3d-W16A-G17A-P18A	GGCTAGCGTTTAAACGGGCCCTCTAGACTCGAGCGGCCGCCACTGTGCTGGATATCTGCAGAATTCAAGATGGCAAAGTTCATGACACCCGTGATCCAGGACAACCCCTCAGGCGCGGCTGCCTGTGCGGTTCCCGAGCAGTTTCGGGATATGCCCTACCAGCCGTTCAGCAAAGGAGATCGGCTAGGAAAGGTTGCAGACTGGACAGGAGCCACATACCAAGATAAGAGGTACACAAATAAGTACTCCTCTCAGTTTGGTGGTGGAAGTCAATATGCTTATTTCCATGAGGAGGATGAAAGTAGCTTCCAGCTGGTGGATACAGCGCGCACACAGAAGACGGCCTACCA

Supplementary Table 6. List of gene strings, Related to STAR Methods section.