

## Supplementary Information

### Properties of the ternary complex formed by yeast eIF4E, p20 and mRNA

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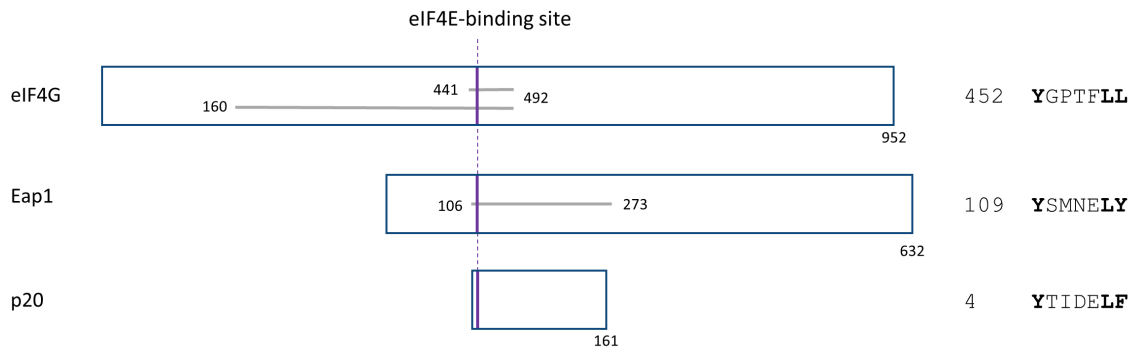
**Supp. Fig.1:**

S1a) Amino acid sequence of yeast p20 (P12962) showing the canonical eIF4E-binding site (aa 4-10; marked in green) and arginine (aa55-57) or histidine residues (aa60-62) that were mutated to leucine in this work (marked in cyan). The N-terminal 18aa p20 peptide used in this work is underlined.

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1  MIKYYTIDELLF QLKPSLTLEV NFDAVEFRAI IEKVKQLQHL KEEEFNSHHV GHFGRRRSSH
61  HHGRPKIKHN KPKVTTSDG WCTFEAKKKG SGEDDEEETE TTPSTVPPVA TIAQETLKVK
121 PNNKNISSNR PADTRDIVAD KPILGFNAFA ALESEDEDDE A*
  
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S1b) Schematic depiction of yeast eIF4G, Eap1 and p20 proteins. The canonical YxxxLL/Y/F eIF4E-binding motif found in each protein is given on the right and its position is indicated by a vertical purple bar. The amino acids corresponding to the eIF4G and Eap1 peptides used in this study are shown as grey horizontal lines.



S1c) Sequence comparison of yeast eIF4E native sequence (top line) vs. codon optimized sequence (DAPCEL; bottom line)

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YEAST_eIF4E      --ATGTCGGTTGAAGAAGTTAGCAAGAAGTTGAAGAAAACGTTTCAGTCGATGATACC
eIF4E_DAPCEL    ATGGGCAGCGTTGAAGAAGTCTCCAAAAAATTTGAAGAAAACGTTAGCGTAGATGATACC
                ***** ** * * ***** ** * * *****

YEAST_eIF4E      ACAGCTACTCCAAAGACTGTTTTAAGTGACAGTGCTCACTTCGATGTCAAGCACCCATTG
eIF4E_DAPCEL    ACCGCGACCCGAAAACCGTTTTAAGCGACAGCGCGCACCTTCGATGTAAAACACCCGTTA
                ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

YEAST_eIF4E      AACACCAAATGGACTTTATGGTACACAAAGCCAGCCGTCGATAAATCTGAGTCGTGGTCT
eIF4E_DAPCEL    AATACTAAATGGACCTTATGGTACACCAAACCGGCTGTAGATAAAAGCGAGTCGTGGAGC
                ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

YEAST_eIF4E      GATCTATTACGTCCTGTCCTTCCAAACTGTTGAAGAATTTGGGCTATCATTCAA
eIF4E_DAPCEL    GATTTGTTAAGACCCGTAACCAGCTTCCAGACCGTTGAAGAATTTGGGCGATCATTCAG
                * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

YEAST_eIF4E      AATATTCCTGAGCCACACGAACTACCATTGAAATCAGATTACCACGTCTTCGGTAATGAC
eIF4E_DAPCEL    AATATTCGGAGCCGACGAATTGCCGTTAAAAGCGATTACCACGTATTCAGAAATGAC
                ***** * * * * * * * * * * * * * * * * * * * * * * *

YEAST_eIF4E      GTTAGACCTGAATGGGAAGATGAAGCCAATGCTAAAGGTGGTAAATGGTCTTTCCAACTT
eIF4E_DAPCEL    GTTCGTCCTGAATGGGAAGATGAAGCTAATGCGAAAAGGTGGTAAATGGAGCTTCCAGTTG
                * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

YEAST_eIF4E      AGAGGAAAAGGTGCTGATATTGATGAATTATGGCTAAGAAGCTTTACTAGCAGTTATTGGT
eIF4E_DAPCEL    CGTGGAAAAGGTGCGGATATTGATGAATTATGGTTGCGTACCTTATTGGCTGTTATTGGT
                * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
  
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YEAST_eIF4E      GAAACAATTGATGAAGACGACTCCCAAATTAACGGTGTCTGTTTTAAGCATTAGAAAAGGT
eIF4E_DAPCEL    GAAACCATTGATGAAGACGACGCCAGATTAACGGTGTAGTTTTATCCATTTCGTAAGGT
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*****

YEAST_eIF4E      GGTAACAAGTTTGCCTTATGGACTAAATCTGAAGCAAAGAACCCTATTGAGAATTGGT
eIF4E_DAPCEL    GGTAATAAATTTGCTTTATGGACCAAAGCGAAGACAAAGAACCGTTGTACGTATTGGT
*****
*****
*****

YEAST_eIF4E      GGTAAATTCAGCAAGTTTTAAAATTAACCGATGACGGGCATTTGGAATTCCTTCCACAT
eIF4E_DAPCEL    GGTAAATTCAAACAGGTTTTAAAATTAACCGATGACGGGCATTTAGAATTTTTCCGCAT
*****
*****
*****

YEAST_eIF4E      TCCAGTGCCAATGGTAGACACCCTCAACCATCAATCACCTTGTA
eIF4E_DAPCEL    AGCAGCGCTAATGGTCGTCACCCGACCCGAGCATCACTTTATA
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*****

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S1d) Sequence comparison of yeast p20 native sequence (top line) vs. codon optimized sequence (DAPCEL; bottom line)

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YEAST_p20      ATGATCAAGTATACTATCGATGAGCTTTTTCAACTGAAGCCAAGTTAACTTTGGAAGTT
p20_DAPCEL    ATGATCAAATATAACCATCGATGAGTTGTTTTAGCTCAAACCGAGCTTAACCTTAGAAGTT
*****

YEAST_p20      AATTTTCGATGCGGTGGAATTTAGAGCCATCATTGAAAAAGTTAAGCAATTGCAACACTTG
p20_DAPCEL    AATTTTCGATGCTGTAGAATTTTCGTGCTATCATCGAAAAAGTTAAACAGTTACAGCACTTA
*****

YEAST_p20      AAAGAGGAAGAGTTTAAACAGTCATCATGTTGGTCATTTTCGGTCGTAGAAGATCTTCCAC
p20_DAPCEL    AAAGAGGAAGAGTTTAAACAGCCATCATGTTGGTCATTTTCGGTAGACGTCGTAGCTCCAC
*****

YEAST_p20      CATCATGGTCGTCCGAAAATTAACACAATAAACCGAAAGTTACCACTGATAGCGATGGT
p20_DAPCEL    CATCATGGTCGTCCGAAAATTAACACAATAAACCGAAAGTTACCACTGATAGCGATGGT
*****

YEAST_p20      TGGTGCACATTTGAAGCCAAGAAGAAGGGTAGTGGAGAAGATGATGAAGAAGAAACAGAA
p20_DAPCEL    TGGTGCACCTTTGAAGCTAAAAAAAAGGTAGCGGGGAAGATGATGAAGAAGAAACCGAA
*****

YEAST_p20      ACCACACCAACTTCTACTGTGCCAGTTGCTACCATTGCCCAAGAACTTTAAAAGTCAAG
p20_DAPCEL    ACTACCCGACCAGCACCGTACCGGTTGCGACTATTGCTCAGAAACCTTAAAAGTAAAA
** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** **

YEAST_p20      CCAAATAACAAAATATTTCTTCCAACAGACCTGCTGATACCAGAGATATTGTTGCGGAC
p20_DAPCEL    CCGAATAACAAAATATCTCTTCCAACCGCCCGCGGATACTCGTGATATTGTTGCTGAC
** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** * ** ** ** ** ** ** ** *

YEAST_p20      AAGCCAATTCTTGGTTTCAACGCATTTGCTGCTTTGGAAAGTGAAGACGAAGACGACGAA
p20_DAPCEL    AAACCGATTTTGGGTTTCAATGCTTTTGGCGGCTTAGAAAGCGAAGACGAAGACGACGAA
** ** ** * ** ** ** ** ** ** ** ** ** ** ** ** ** * ** ** ** * ** ** * ** ** * ** ** *

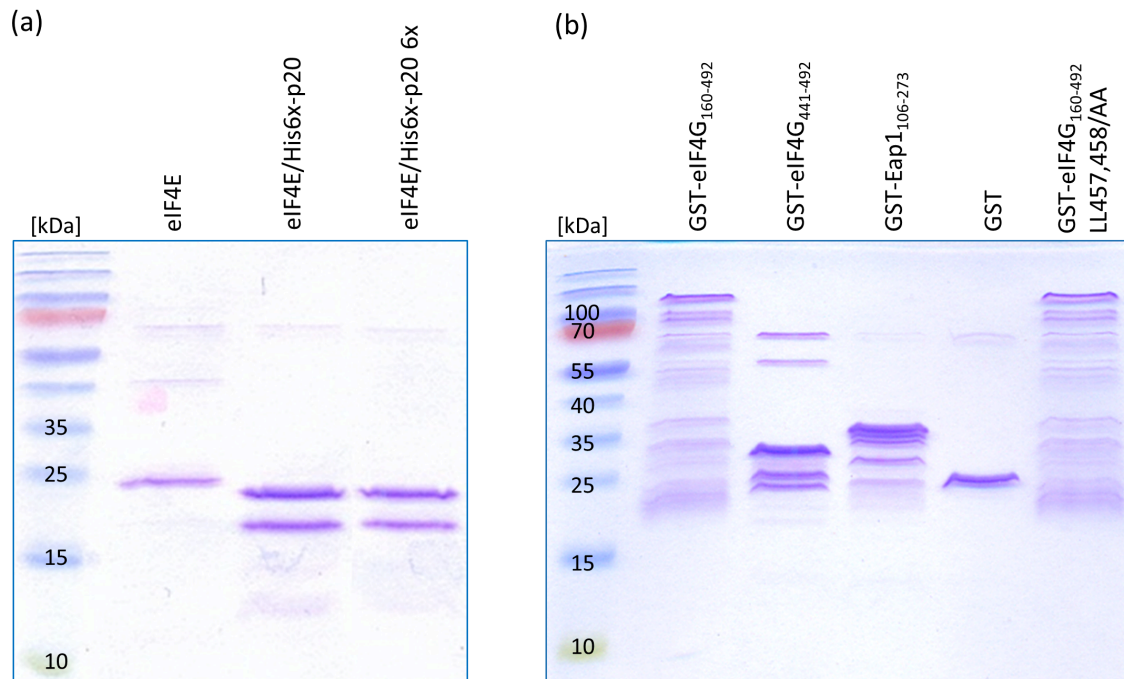
YEAST_p20      GCATAA
p20_DAPCEL    GCTTGA
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S1e) Nucleotide sequence of RNAs used in this work:

3'FAM labeled 40 nt SSA1 RNA (Microsynth AG), used for MicroScale Thermophoresis™ or Electrophoretic Mobility Shift Assay measurements	GUAUUACAAGAAACAAAAUUUCAAGUAAUUAACAG AUAUU
<i>In vitro</i> transcribed, m <sup>7</sup> GTP- or m <sup>7</sup> GpppG-capped 64 nt SSA1 5'UTR RNA, used for MicroScale Thermophoresis™ or Electrophoretic Mobility Shift Assay measurements	GGGAACAAAAGCUGGUAUUAAGAAUUACAAGAAA CAAAAUUUCAAGUAAUUAACAGUAAUUAAC
"Average" 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for <i>in vitro</i> translation assays	GGGAACAAAAGCUGGAGCUCGCCCGGGCUGUUCUA GCCACCA <b>AUGG</b>

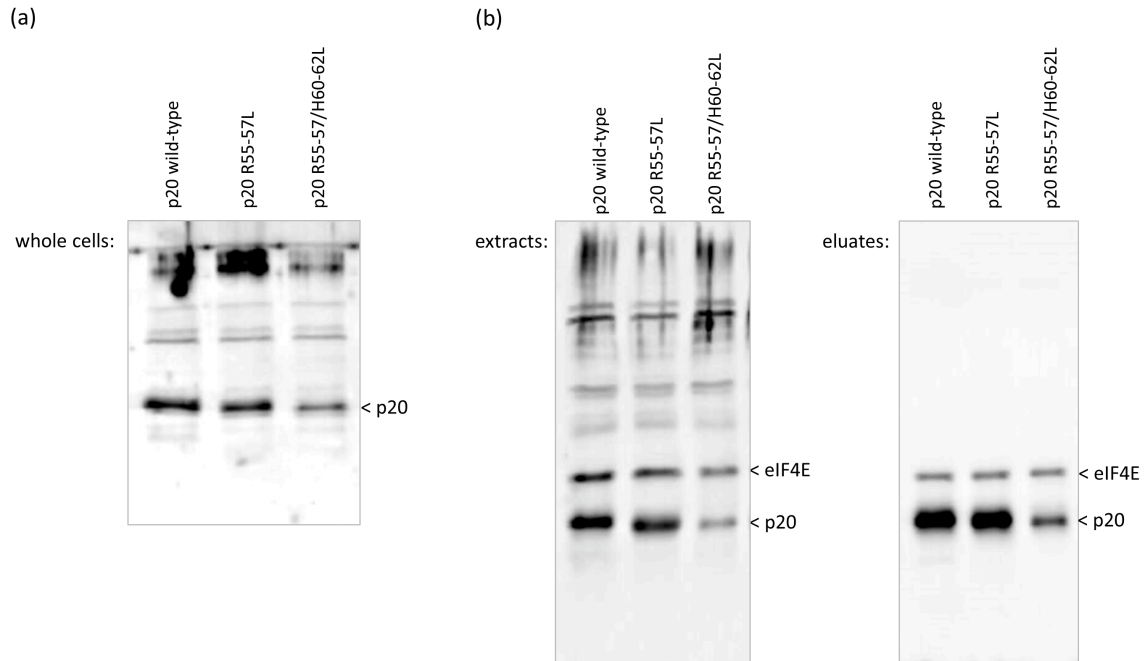
PMA1 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for <i>in vitro</i> translation assays	GGGAACAAAAGCUGGAGCUCGCCCGGGACACCAAU AGUGAAAUCUUUUUUUCUUAUAUCUACAAAAA CUUUUUUUUCUACAAAAACUUUUUUUUUCUAUCA ACCUCGUUGAUAUUUUUUUCUUUAACAAUCGUUA AUAUUAAUUAAUUGGAAAAUAACCAUUUUUCUC UCUUUUUAUCACACAUUCAAAAAGAAAAGAAAAA UAUACCCCAGCUAGUUAAAGAAAUAUUGAAAAG AAUAAGAAGUAAGAAAGAUUUAAUUUAUCAACAA UAUCAAUCCA <b>AUGG</b>
GRE1 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for <i>in vitro</i> translation assays	GGGAACAAAAGCUGGAGCUCGCCCGGGGAGCCAAA CACUACCGCAUAAAAGCUAAGUACGAAUAACAAUU AAGAACC <b>AUGG</b>
SSA1 5'UTR fused to Renilla Luciferase ORF (AUG marked in bold), used for <i>in vitro</i> translation assays	GGGAACAAAAGCUGGUAUAUCAAGAAUUACAAGAAA CAAAAAUUCAGUAAAUAACAGAUAAUACC <b>AUGG</b>



**Supp. Fig.2:** Coomassie stained SDS-PAGE (20%)

(S2a) 0.75  $\mu$ g purified His6x-eIF4E alone (lane 1), 2.0  $\mu$ g co-expressed and purified eIF4E/His6x-p20 complex (lane 2), and 2.0  $\mu$ g co-expressed and purified eIF4E/His6x-p20 6x mutant complex (lane 3)

(S2b) 4  $\mu$ g GST-eIF4G<sub>160-492</sub> (lane 1), 4  $\mu$ g GST-eIF4G<sub>441-492</sub> (lane 2), 4  $\mu$ g GST-Eap1<sub>106-273</sub> (lane 3), 4  $\mu$ g GST (lane 4), and 4  $\mu$ g GST-eIF4G<sub>160-492</sub> LL457,458/AA mutant (lane 5).



**Supp. Fig. 3:** Full-length Western blots (sections of which are presented in Figure 2d). Blots were stained with polyclonal antibodies against eIF4E or p20 as described in Methods.

S3a) Western blot of  $\frac{1}{2}$  OD<sub>600</sub> whole yeast cells carrying p20 knockout (RH2585  $\Delta$ p20) and expressing p20 wild type, R55-57L (3x) or R55-57L, H60-62L (6x) mutants.

S3b) Western blots of input extracts (10  $\mu$ g loaded on blot) used for m<sup>7</sup>GDP-Sepharose pulldown (left panel) and eluates (right panel) for p20 wild type, R55-57L (3x) and R55-57L, H60-62L (6x) mutants.