A yeast system for discovering optogenetic inhibitors of eukaryotic translation initiation

Supplementary Information

Rationally designed opto-4EBP sequences screened in pRS316

cPYP-C_0

```
         10          20          30          40          50          60
MKGDSYWVFV KRVGTRIIYD RKFLLDRRNM EHVAFGSEDI ENTLAKMDDG QLDGLAFGAI
         70          80          90         100         110         120
QLDGDGNILQ YNAAEGDITG RDPKQVIGKN FFKDVAPCTD SPEFYGKFKE GVASGNLNTM
        130         140
FEYTFDYQAT PTKVKVHMKK ALS
```

cPYP-C_1

```
         10          20          30          40          50          60
MKGDSYWVFV KRVGTRIIYD RKFLLDRRNA PMAQAPPCHL PNIPGVTSPM EHVAFGSEDI
         70          80          90         100         110         120
ENTLAKMDDG QLDGLAFGAI QLDGDGNILQ YNAAEGDITG RDPKQVIGKN FFKDVAPCTD
        130         140         150         160
SPEFYGKFKE GVASGNLNTM FEYTFDYQAT PTKVKVHMKK ALS
```

cPYP-C_1N_1

```
         10          20          30          40          50          60
MKGDSYWVFV KRVDYCTAPG GTLFSTAPGG TRIIYDRKFL LDRRNAPMAQ APPCHLPNIP
         70          80          90         100         110         120
GVTSPMEHVA FGSEDIENTL AKMDDGQLDG LAFGAIQLDG DGNILQYNAA EGDITGRDPK
        130         140         150         160         170
QVIGKNFFKD VAPCTDSPEF YGKFKEGVAS GNLNTMFEYT FDYQATPTKV KVHMKKALS
```

RsLOV-4EBP2-RsLOV (RBPR)

```
         10          20          30          40          50          60
MDQKQFEKIR AVFDRSGVAL TLVDMSLPEQ PVVLANPPFL RMTGYTEGQI LGFNCRFLQR
         70          80          90         100         110         120
GDENAQARAD IRDALKLGRE LQVVLRNYRA NDEPFDNLLF LHPVGGRPDA PDYFLGSQFE
        130         140         150         160         170         180
LGRSGNSEEA AAAGHAGALT GELARIGTVA ARLEMDSRRH LAQAAAALVR AWERRGDYCT
        190         200         210         220         230         240
APGGTLFSTA PGGTRIIYDR KFLLDRRNAP MAQAPPCHLP NIPGVTSPMD QKQFEKIRAV
        250         260         270         280         290         300
FDRSGVALTL VDMSLPEQPV VLANPPFLRM TGYTEGQILG FNCRFLQRGD ENAQARADIR
        310         320         330         340         350         360
DALKLGRELQ VVLRNYRAND EPFDNLLFLH PVGGRPDAPD YFLGSQFELG RSGNSEEAAA
        370         380         390         400
AGHAGALTGE LARIGTVAAR LEMDSRRHLA QAAAALVRAW ERRG
```

4EBP2-RsLOV (BPR)

```
        10         20         30         40         50         60
MDYCTAPGGT LFSTAPGGTR IIYDRKFLLD RRNAPMAQAP PCHLPNIPGV TSPMDQKQFE
        70         80         90        100        110        120
KIRAVFDRSG VALTLVDMSL PEQPVVLANP PFLRMTGYTE GQILGFNCRF LQRGDENAQA
       130        140        150        160        170        180
RADIRDALKL GRELQVVLRN YRANDEPFDN LLFLHPVGGR PDAPDYFLGS QFELGRSGNS
       190        200        210        220
EEAAAAGHAG ALTGELARIG TVAARLEMDS RRHLAQAAAA LVRAWERRG
```

RsLOV-4EBP2 (RBP)

```
        10         20         30         40         50         60
MDQKQFEKIR AVFDRSGVAL TLVDMSLPEQ PVVLANPPFL RMTGYTEGQI LGFNCRFLQR
        70         80         90        100        110        120
GDENAQARAD IRDALKLGRE LQVVLRNYRA NDEPFDNLLF LHPVGGRPDA PDYFLGSQFE
       130        140        150        160        170        180
LGRSGNSEEA AAAGHAGALT GELARIGTVA ARLEMDSRRH LAQAAAALVR AWERRGDYCT
       190        200        210        220
APGGTLFSTA PGGTRIIYDR KFLLDRRNAP MAQAPPCHLP NIPGVTSP
```

Dronpa145K-4EBP2-Dronpa145N

```
        10         20         30         40         50         60
MSVIKPDMKI KLRMEGAVNG HPFAIEGVGL GKPFEGKQSM DLKVKEGGPL PFAYDILTTV
        70         80         90        100        110        120
FCYGNRVFAK YPENIVDYFK QSFPEGYSWE RSMNYEDGGI CNATNDITLD GDCYIYEIRF
       130        140        150        160        170        180
DGVNFPANGP VMQKRTVKWE PSTEKLYVRD GVLKGDVNMA LSLEGGGHYR CDFKTTYKAK
       190        200        210        220        230        240
KVVQLPDYHF VDHHIEIKSH DKDYSNVNLH EHAEAHSELP RQAKTRIIYD RKFLLDRRNA
       250        260        270        280        290        300
PMAQAPPCHL PNIPGVTSPG MSVIKPDMKI KLRMEGAVNG HPFAIEGVGL GKPFEGKQSM
       310        320        330        340        350        360
DLKVKEGGPL PFAYDILTTV FCYGNRVFAK YPENIVDYFK QSFPEGYSWE RSMNYEDGGI
       370        380        390        400        410        420
CNATNDITLD GDCYIYEIRF DGVNFPANGP VMQKRTVKWE PSTENLYVRD GVLKGDVNMA
       430        440        450        460        470        480
LSLEGGGHYR CDFKTTYKAK KVVQLPDYHF VDHHIEIKSH DKDYSNVNLH EHAEAHSELP
       490        500
RQAKGLENLY FQGLEHHHHH H
```

Dronpa145N-4EBP2-Dronpa145N

```
        10         20         30         40         50         60
MSVIKPDMKI KLRMEGAVNG HPFAIEGVGL GKPFEGKQSM DLKVKEGGPL PFAYDILTTV
        70         80         90        100        110        120
FCYGNRVFAK YPENIVDYFK QSFPEGYSWE RSMNYEDGGI CNATNDITLD GDCYIYEIRF
       130        140        150        160        170        180
DGVNFPANGP VMQKRTVKWE PSTENLYVRD GVLKGDVNMA LSLEGGGHYR CDFKTTYKAK
       190        200        210        220        230        240
KVVQLPDYHF VDHHIEIKSH DKDYSNVNLH EHAEAHSELP RQAKTRIIYD RKFLLDRRNA
       250        260        270        280        290        300
PMAQAPPCHL PNIPGVTSPG MSVIKPDMKI KLRMEGAVNG HPFAIEGVGL GKPFEGKQSM
       310        320        330        340        350        360
DLKVKEGGPL PFAYDILTTV FCYGNRVFAK YPENIVDYFK QSFPEGYSWE RSMNYEDGGI
```

```
       370        380        390        400        410        420
CNATNDITLD GDCYIYEIRF DGVNFPANGP VMQKRTVKWE PSTENLYVRD GVLKGDVNMA
       430        440        450        460        470        480
LSLEGGGHYR CDFKTTYKAK KVVQLPDYHF VDHHIEIKSH DKDYSNVNLH EHAEAHSELP
       490        500
RQAKGLENLY FQGLEHHHHH H
```

Split-4EBP2

```
        10         20         30         40         50         60
MKGTRIIYDR KFLLDRRNGS LATTLERIEK NFIITDPRLP DNPIIFASDS FLQLTEYSRE
        70         80         90        100        110        120
EILGRNCRFL QGPETDRATV RKIRDAIDNQ TEVTVQLINY TKSGKKFWNL FHLQPMRDQK
       130        140        150        160        170        180
GDVQYFIGVQ LDGTEHVRDA AEREAVMLIK KTAEEIDEAA GSAPMAQAPP CHLPNIPGVT
       190
SPLEHHHHHH
```

Split-4EBP2(W)

```
        10         20         30         40         50         60
MRYSKVDLLA LRYSPLSQTG SLATTLERIE KNFIITDPRL PDNPIIFASD SFLQLTEYSR
        70         80         90        100        110        120
EEILGRNCRF LQGPETDRAT VRKIRDAIDN QTEVTVQLIN YTKSGKKFWN LFHLQPMRDQ
       130        140        150        160        170        180
KGDVQYFIGV QLDGTEHVRD AAEREAVMLI KKTAEEIDEA AGSELEGRLR RMNIWRTGSL

EHHHHHH
```

LOV-4EBP2-Merge

```
        10         20         30         40         50         60
MLATTLERIE KNFIITDPRL PDNPIIFASD SFLQLTEYSR EEILGRNCRF LQGPETDRAT
        70         80         90        100        110        120
VRKIRDAIDN QTEVTVQLIN YTKSGKKFWN LFHLQPMRDQ KGDVQYFIGV QLDGTEHVRD
       130        140        150
AAEYEGVMLI KKTAENIDEA AKELPDAIPG VTDLWANH
```

LOV-4EBP2

```
        10         20         30         40         50         60
MKLATTLERI EKNFVITDPR LPDNPIIFAS DSFLQLTEYS REEILGRNCR FLQGPETDRA
        70         80         90        100        110        120
TVRKIRDAID NQTEVTVQLI NYTKSGKKFW NLFHLQPMRD QKGDVQYFIG VQLDGTEHVR
       130        140        150        160        170        180
DAAEREAVML IKKTAEEIDE AARIIYDRKF LLDRRNAPMA QAPPCHLPNI PGVTSPGTLI
       190
EDSKVEVNNL NNLNNH
```

LOV-4EBP2-v2

```
        10         20         30         40         50         60
MKLATTLERI EKNFIITDPR LPDNPIIFAS DSFLQLTEYS REEILGRNCR FLQGPETDRA
        70         80         90        100        110        120
TVRKIRDAID NQTEVTVQLI NYTKSGKKFW NLFHLQPMRD QKGDVQYFIG VQLDGTEHVR
       130        140        150        160        170
GTRIIYDRKF LLDRRNDAAE REAVMLIKKT AENIDEAAKE LPDANLRPED LWANH
```

cLOV-wt4EBP2

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML IKKTAEEIDE AAMSSSAGSG HQPSQSRAIP
        70         80         90        100        110        120
TRTVAISDAA QLPHDYCTTP GGTLFSTTPG GTRIIYDRKF LLDRRNSPMA QTPPCHLPNI
       130        140        150        160        170        180
PGVTSPGTLI EDSKVEVNNL NNLNNHDRKH AVGDDAQFEM DIEFLATTLE RIEKNFIITD
       190        200        210        220        230        240
PRLPDNPIIF ASDSFLQLTE YSREEILGRN CRFLQGPETD RATVRKIRDA IDNQTEVTVQ
       250        260
LINYTKSGKK FWNLFHLQPM RDQ
```

cLOV-wt4EBP2-I31A

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML AKKTAEEIDE AAMSSSAGSG HQPSQSRAIP
        70         80         90        100        110        120
TRTVAISDAA QLPHDYCTTP GGTLFSTTPG GTRIIYDRKF LLDRRNSPMA QTPPCHLPNI
       130        140        150        160        170        180
PGVTSPGTLI EDSKVEVNNL NNLNNHDRKH AVGDDAQFEM DIEFLATTLE RIEKNFIITD
       190        200        210        220        230        240
PRLPDNPIIF ASDSFLQLTE YSREEILGRN CRFLQGPETD RATVRKIRDA IDNQTEVTVQ
       250        260
LINYTKSGKK FWNLFHLQPM RDQ
```

cLOV-Ntr4EBP2

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML IKKTAEEIDE AARIIYDRKF LLDRRNSPMA
        70         80         90        100        110        120
QTPPCHLPNI PGVTSPGTLI EDSKVEVNNL NNLNNHDRKH AVGDDAQFEM DIEFLATTLE
       130        140        150        160        170        180
RIEKNFIITD PRLPDNPIIF ASDSFLQLTE YSREEILGRN CRFLQGPETD RATVRKIRDA
       190        200        210
IDNQTEVTVQ LINYTKSGKK FWNLFHLQPM RDQ
```

cLOV-Ntr4EBP2-I31A

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML AKKTAEEIDE AARIIYDRKF LLDRRNSPMA
        70         80         90        100        110        120
QTPPCHLPNI PGVTSPGTLI EDSKVEVNNL NNLNNHDRKH AVGDDAQFEM DIEFLATTLE
       130        140        150        160        170        180
RIEKNFIITD PRLPDNPIIF ASDSFLQLTE YSREEILGRN CRFLQGPETD RATVRKIRDA
       190        200        210
IDNQTEVTVQ LINYTKSGKK FWNLFHLQPM RDQ
```

cLIPS1 in pET24b vector

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML IKKTAEEIDE AARIIYDRKF LLDRRNAPMA
        70         80         90        100        110        120
QAPPCHLPNI PGVTSPGEFL ATTLERIEKN FIITDPRLPD NPIIFASDSF LQLTEYSREE
       130        140        150        160        170        180
ILGRNCRFLQ GPETDRATVR KIRDAIDNQT EVTVQLINYT KSGKKFWNLF HLQPMRDQKL
       190
AAALEHHHHH H
```

cLIPS1^{AA} in pET24b vector

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML IKKTAEEIDE AARIIYDRKF AADRRNAPMA
        70         80         90        100        110        120
QAPPCHLPNI PGVTSPGEFL ATTLERIEKN FIITDPRLPD NPIIFASDSF LQLTEYSREE
       130        140        150        160        170        180
ILGRNCRFLQ GPETDRATVR KIRDAIDNQT EVTVQLINYT KSGKKFWNLF HLQPMRDQKL
       190
AAALEHHHHH H
```

cLIPS2 in pET24b vector

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML IKKTAEEIDE AARIIYDRKF LLDRRNAPMA
        70         80         90        100        110        120
QAPPCHLPNI PGVTSPGEFL ATTLERIGVT SPGEFLATTL ERIEKNFIIT DPRLPDNPII
       130        140        150        160        170        180
FASDSFLQLT EYSREEILGR NCRFLQGPET DRATVRKIRD AIDNQTEVTV QLINYTKSGK
       190        200
KFWNLFHLQP MRDQKLAAAL EHHHHHH
```

cLIPS2^{AA} in pET24b vector

```
        10         20         30         40         50         60
MKGDVQYFIG VQLDGTEHVR DAAEREAVML IKKTAEEIDE AARIIYDRKF AADRRNAPMA
        70         80         90        100        110        120
QAPPCHLPNI PGVTSPGEFL ATTLERIGVT SPGEFLATTL ERIEKNFIIT DPRLPDNPII
       130        140        150        160        170        180
FASDSFLQLT EYSREEILGR NCRFLQGPET DRATVRKIRD AIDNQTEVTV QLINYTKSGK
       190        200
KFWNLFHLQP MRDQKLAAAL EHHHHHH
```

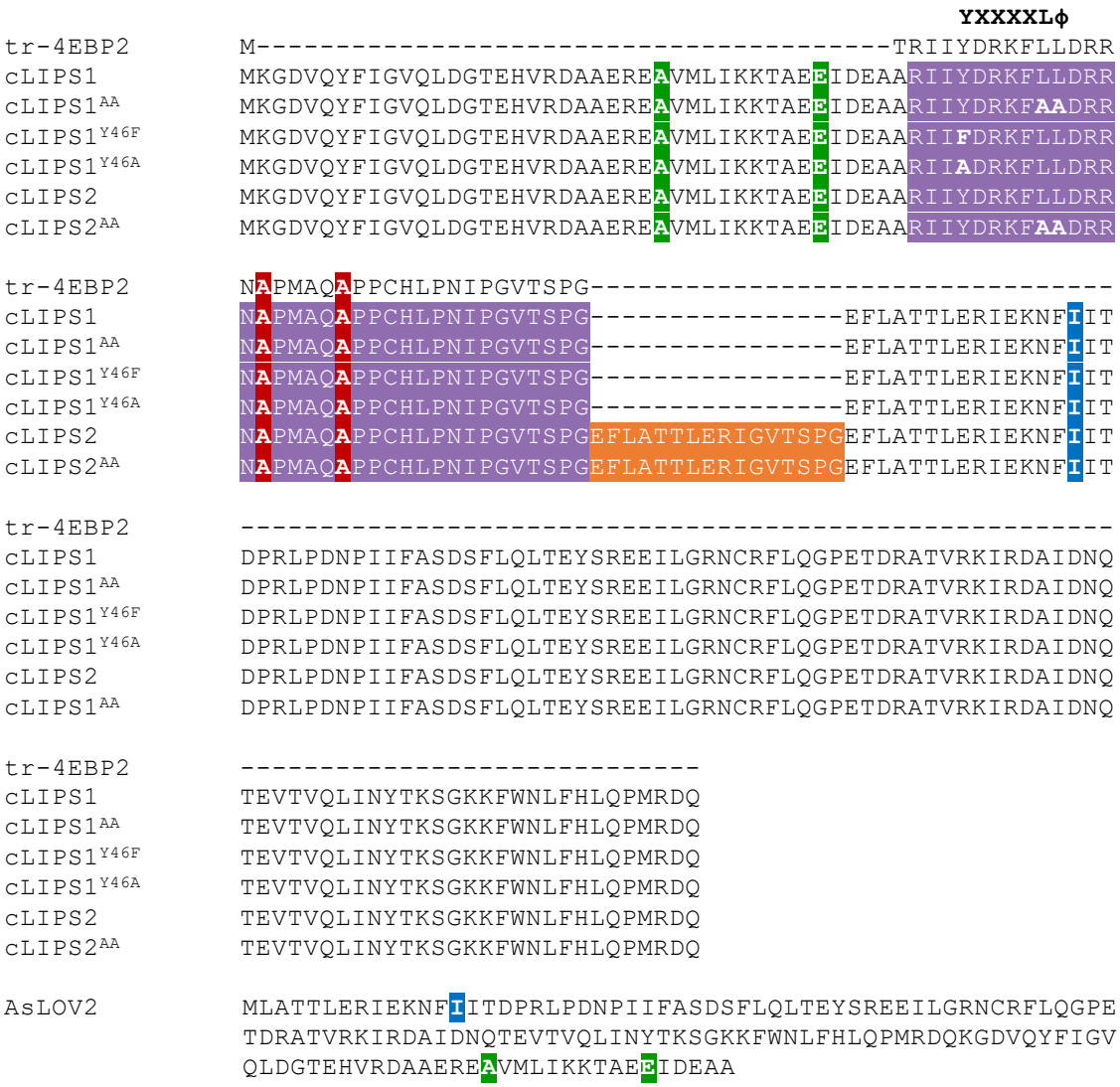
GB1-eIF4E in pET24b vector

```
        10         20         30         40         50         60
MKHHHHHHMQ YKLILNGKTL KGETTTEAVD AATAEKVFKQ YANDNGVDGE WTYDDATKTF
        70         80         90        100        110        120
TVTEGENLYF QGGMATVEPE TTPTPNPPTT EEEKTESNQE VANPEHYIKH PLQNRWALWF
       130        140        150        160        170        180
FKNDKSKTWQ ANLRLISKFD TVEDFWALYN HIQLSSNLMP GCDYSLFKDG IEPMWEDEKN
       190        200        210        220        230        240
KRGGRWLITL NKQQRRSDLD RFWLETLLCL IGESFDDYSD DVCGAVVNVR AKGDKIAIWT
       250        260        270        280        290
TECENREAVT HIGRVYKERL GLPPKIVIGY QSHADTATKS GSTTKNRFVV
```

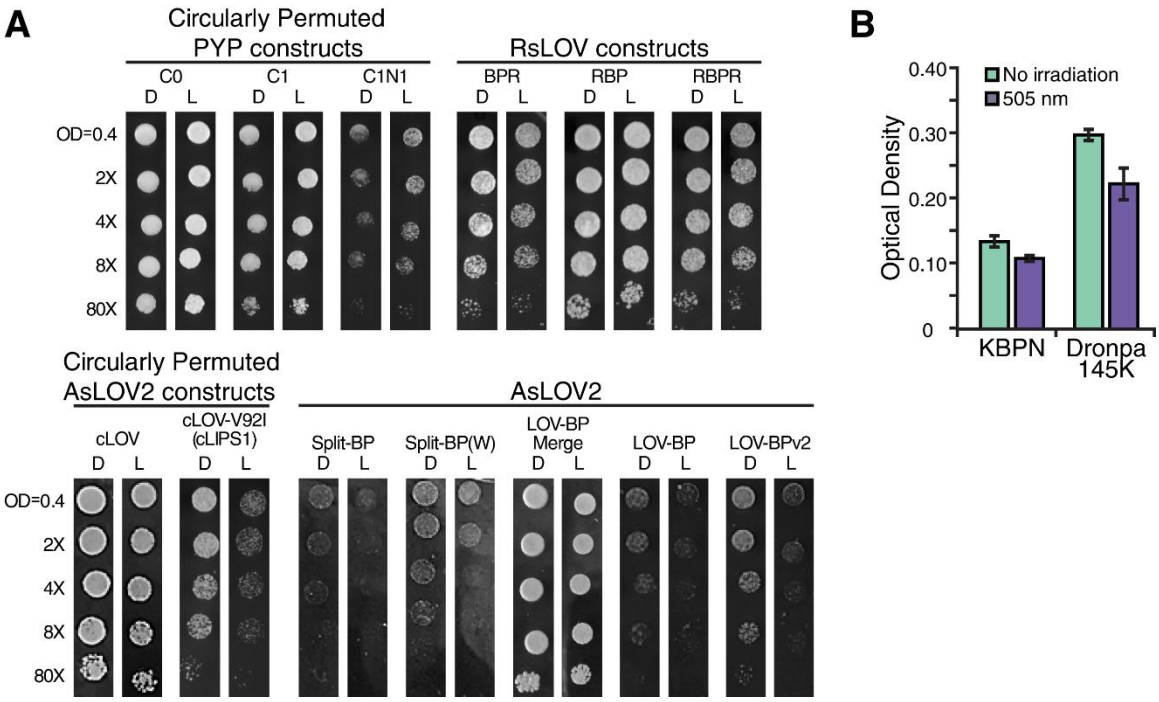
Wt-4EBP2 (Gift from Alaji Bah in Forman-Kay lab):

```
        10         20         30         40         50         60
MSSSAGSGHQ PSQSRAIPTR TVAISDAAQL PHDYCTTPGG TLFSTTPGGT RIIYDRKFLL
        70         80         90        100        110        120
DRRNSPMAQT PPCHLPNIPG VTSPGTLIED SKVEVNNLNN LNNHDRKHAV GDDAQFEMDI
```

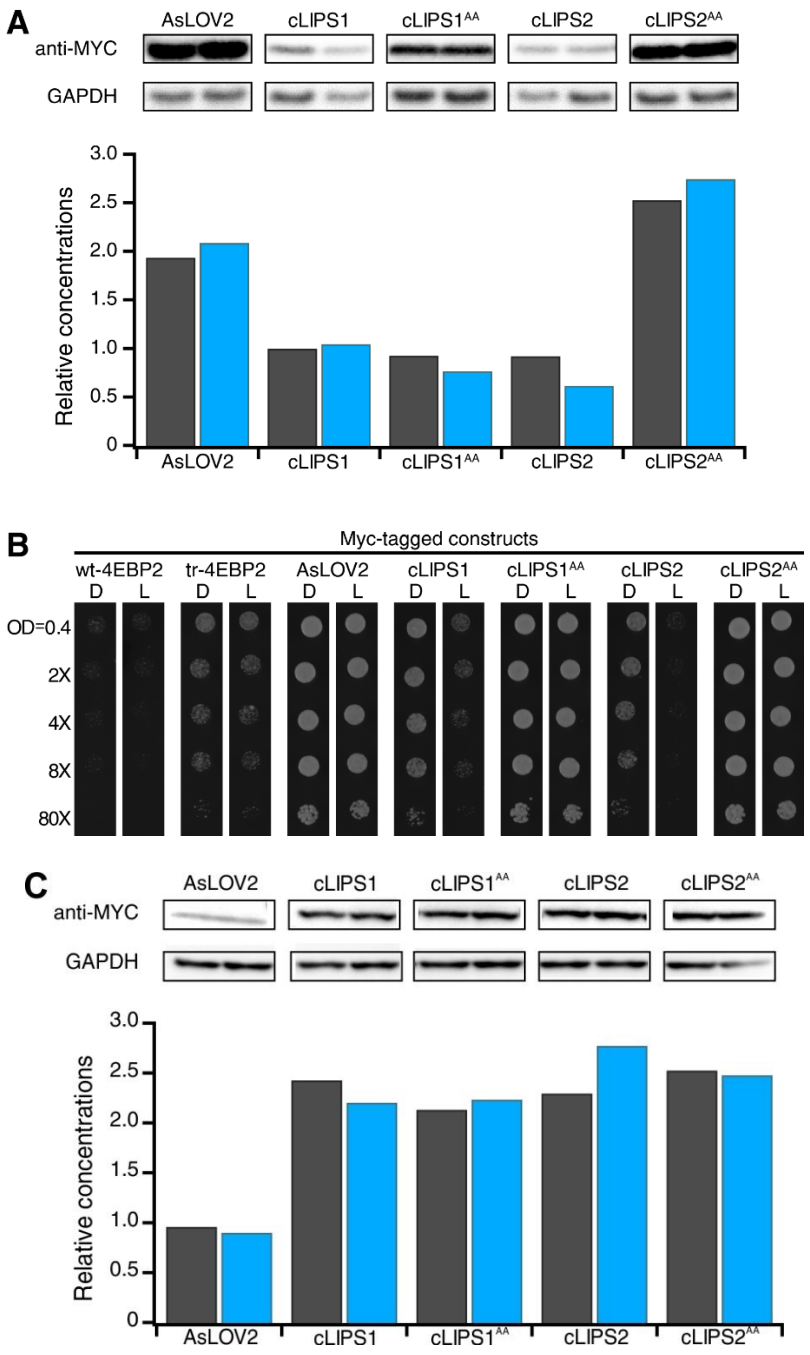
Supplementary Figure 1. Protein sequences for all constructs screened in Jo56 yeast. Also included are the cLIPS, GB1-eIF4E, and wt-4EBP2 protein sequences used for *in vitro* assays.



Supplementary Figure 2. An alignment of the tr-4EBP2, cLIPS1, cLIPS1^{AA}, cLIPS2, cLIPS2^{AA} and AsLOV2 protein sequences. Purple highlights the 4EBP2 insert in the optogenetic constructs. Red highlights indicate where 4EBP2 phosphorylation sites were mutated to alanine. Orange highlights show the duplication that occurred in cLIPS2. Green highlights indicate mutations made in the AsLOV2 sequence to stabilize the dark-state (Strickland et al Nature Methods 7, 623-626, 2010). Blue highlights the mutation made in AsLOV2 to increase the half-life of thermal relaxation (Zoltowski et al, Nature Chemical Biology 5, 827-834, 2009).



Supplementary Figure 3. (A) Yeast growth assay results for all screened opto-4EBP constructs, except Dronpa constructs, in drop test format. Cultures are spotted at the indicated ODs and growth is imaged after 3 days at 30°C under light (L) or dark (D) conditions. Yeast expressing cPYP constructs were grown on plates containing 0.4 µM p-coumaric acid-*S*-thiophenyl ester. (B) Growth of yeast expressing KBPN and the Dronpa145K domain alone (without 4EBP) in liquid culture. These results show that the light intensity required for Dronpa photoswitching (>1 mW/cm^2) causes non-specific inhibition of growth. This can be seen in the growth of the yeast expressing Dronpa145K under 505 nm light. The KBPN construct caused additional growth inhibition although the effect of light alone on growth obscures any potential light-dependent effects of these constructs.

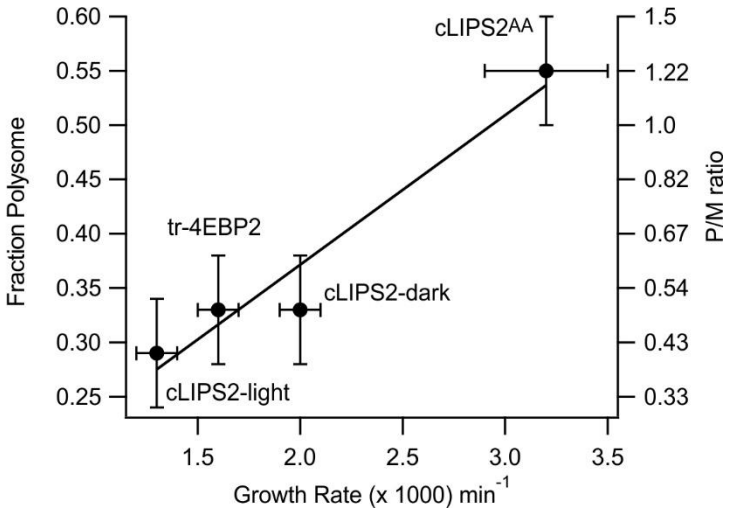


Supplementary Figure 4. (A) Western blot analysis of MYC-tagged constructs from Jo56 yeast cells grown in liquid media under dark (gray) and light (blue) conditions. Relative expression levels are normalized by GAPDH expression. GAPDH levels and total protein levels as measured by stain-free gel analysis [1] (not shown) were directly correlated. Note that variations in the efficiency of yeast lysis caused significant variations in loading between lanes in this blot. (B) Yeast growth assay results for all MYC-tagged constructs in drop test format. Cultures are spotted at the indicated ODs and growth is imaged after 3 days at 30°C under light (L) or dark (D) conditions. (C) Western

blot analysis of MYC-tagged constructs from wild-type yeast cells (expressing yeast 4E) grown in liquid media under dark (gray) and light (blue) conditions. Relative expression levels are normalized by GAPDH expression. (Note the GAPDH control for cLIPS2^{AA} did not image correctly, and the average GAPDH intensity was used instead).

Further information on polysome analysis and growth rates

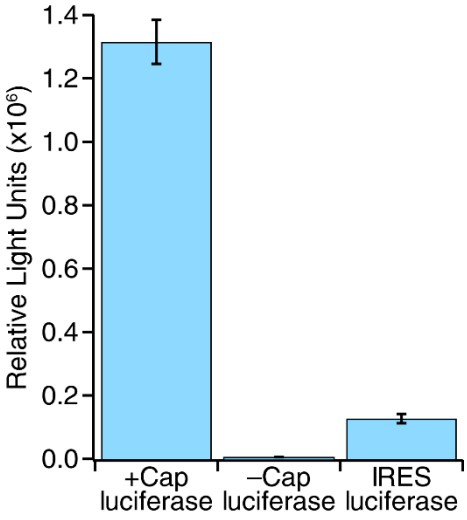
A rudimentary treatment suggests that the average translation rate at a given point in time is linearly related to the fraction of ribosomes bound to mRNA (*e.g.* the fraction of polysomes) [2]. (Note however, recent findings suggest that monosomes can also be translationally active [3]). The translation rate and the growth rate are clearly related and simple theories also suggest a linear relationship (*e.g.* [4]). More detailed studies that have simulated molecular events involved in translation suggest more complex relationships [5, 6], and it is clear that the relationship between growth rate and rate of translation will differ for different proteins [7-9]. Despite these caveats, observed growth rates and observed polysome/monosome (P/M) ratios in the current system were found to be consistent with an approximately linear relationship between the fraction of ribosomes found as polysomes, the overall translation rate, and the growth rate (all measured in early log phase)(Supplementary Figure 5). This correlation, together with the experimental error associated with the polysome analysis means that one would not expect to see a significant difference between the polysome profiles of yeast expressing cLIPS2 under dark vs. light conditions.



Supplementary Figure 5. Observed and calculated parameters for yeast strains tested. Growth rates were calculated as *ln* 2/doubling time (*e.g.* (*ln* 2/220 min) 3.15 *10^{-3} min^{-1}). The observed P/M ratios and calculated fraction polysome ((P/M)/(1+(P/M)) are also shown. The ratio between growth rate and fraction polysome is constant within experimental error.

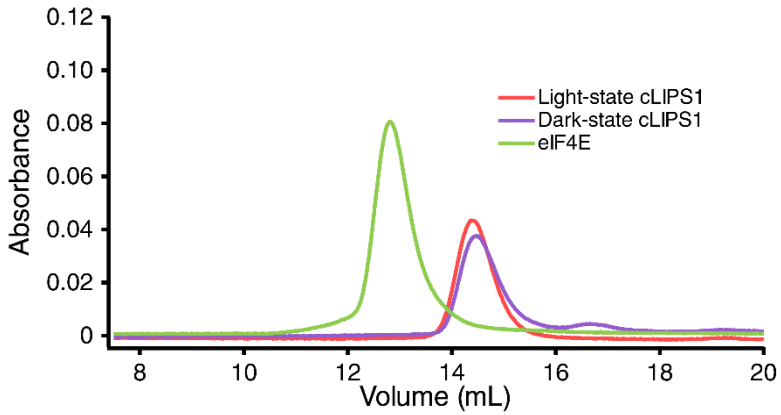
	Expected Masses (Da)	Observed Masses (Da)
cLIPS1	21995.1 Da	21995.0
cLIPS1^{AA}	21910.9 Da	21910.8
cLIPS2	23668.0 Da	23667.9
cLIPS2^{AA}	23583.8 Da	23584.0
GB1-eIF4E	33350.3 Da	33350.9

Supplementary Figure 6. Expected and observed masses of purified proteins obtained by electrospray ionization mass spectrometry.

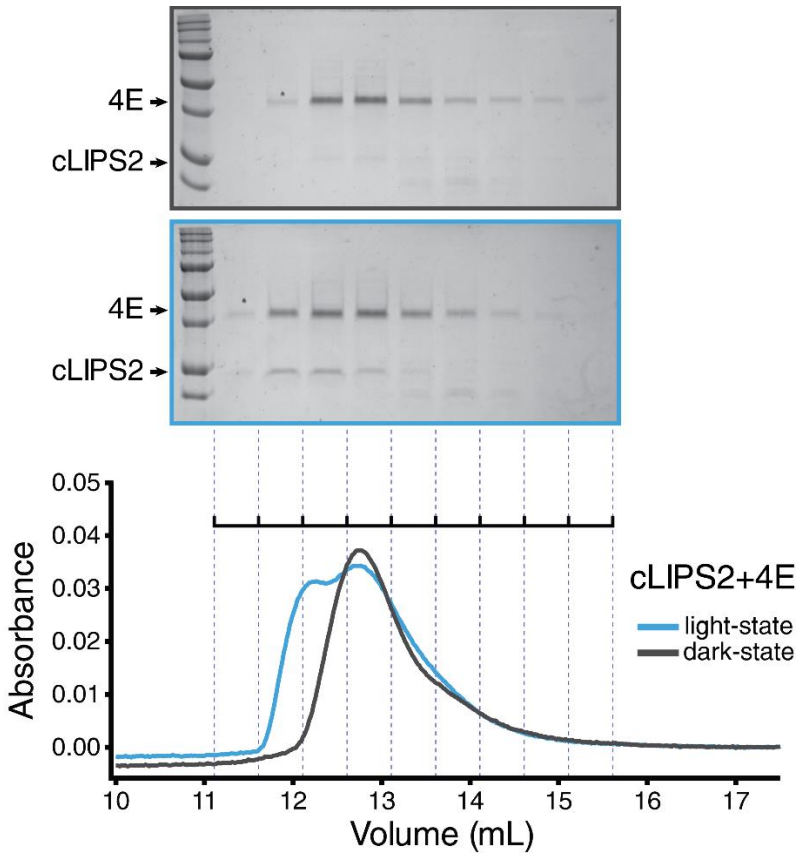


	Average	SD
+Cap luciferase	1314947	69161
–Cap luciferase	5419	513
IRES luciferase	126846	14482

Supplementary Figure 7. Yeast *in vitro* translation assay controls with capped, uncapped, and IRES-mediated Renilla luciferase mRNA. RNA prepared from plasmid 1762 was either left uncapped or capped as described in the methods section.



Supplementary Figure 8. SEC chromatographs of GB1-eIF4E alone (green) or cLIPS1 alone after 1 min blue light irradiation (red) or in the dark (brown). Both GB1-4E and cLIPS1 elute as single peaks that are not overlapped. The peak for cLIPS1 is not substantially affected by light.



Supplementary Figure 9. SEC chromatographs of cLIPS2 (10 µM) mixed with eIF4E (10 µM) in the dark (black lines) or under constant 450 nm light (blue lines). Fractions were collected from both chromatographs and analyzed by SDS-PAGE. A larger complex elutes earlier in the light-state cLIPS2 + eIF4E.

Supplementary References.

1. Rivero-Gutierrez, B., et al., *Stain-free detection as loading control alternative to Ponceau and housekeeping protein immunodetection in Western blotting.* Anal Biochem., 2014. **467**: p. 1-3.
2. Zouridis, H. and V. Hatzimanikatis, *A model for protein translation: polysome self-organization leads to maximum protein synthesis rates.* Biophys. J., 2007. **92**(3): p. 717-30.
3. Heyer, E.E. and M.J. Moore, *Redefining the Translational Status of 80S Monosomes.* Cell, 2016. **164**(4): p. 757-69.
4. Scott, M., et al., *Emergence of robust growth laws from optimal regulation of ribosome synthesis.* Mol. Syst. Biol., 2014. **10**: p. 747.
5. Shah, P., et al., *Rate-limiting steps in yeast protein translation.* Cell, 2013. **153**(7): p. 1589-601.
6. Klumpp, S., Z. Zhang, and T. Hwa, *Growth rate-dependent global effects on gene expression in bacteria.* Cell, 2009. **139**(7): p. 1366-75.
7. Dowling, R.J., et al., *mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs.* Science, 2010. **328**(5982): p. 1172-6.
8. Sonenberg, N. and A.C. Gingras, *The mRNA 5' cap-binding protein eIF4E and control of cell growth.* Curr. Opin. Cell Biol., 1998. **10**(2): p. 268-75.
9. Culjkovic, B., et al., *eIF4E is a central node of an RNA regulon that governs cellular proliferation.* J. Cell Biol., 2006. **175**(3): p. 415-26.