Supplementary information to **eIF4B stimulates eIF4A ATPase and unwinding activities by direct interaction through its 7-repeats region**, Alexandra Z. Andreou, Ulf Harms & Dagmar Klostermeier.

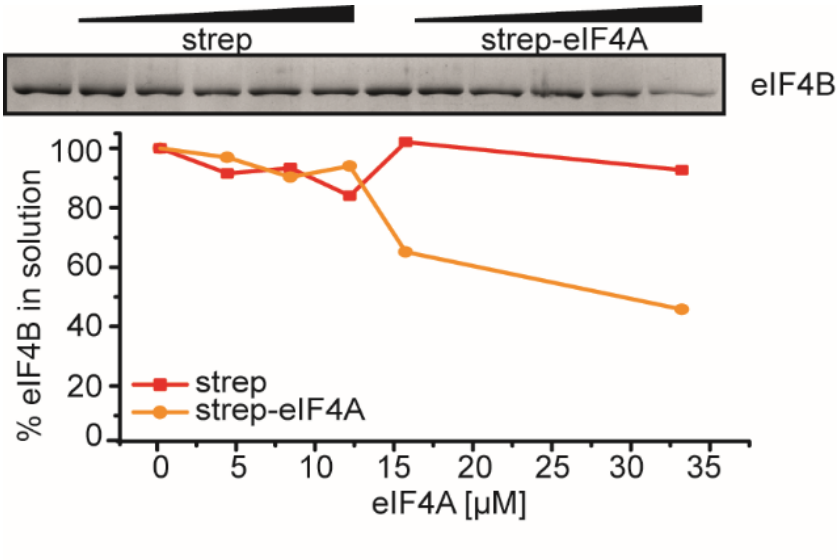
Supplementary Table

Table S1: **Oligonucleotides used in this study**

#	primer	sequence (5'→3')
PB-001	*Tif3_ΔNTD*-FW	CCACCATATGTCCGATAGAAGAGAGGAATACCC
PB-002	*Tif3*_ΔCTD-N	CAGTTTGGTAAGTAACCTCAAC
PB-003	*Tif3*_ΔCTD-C	GTTGAGGTTACTTACCAAACTG
PB-004	*Tif3*_ΔRRM-N	GCACCTCCCCCTCTTCTTGGAGGGTATTCCTCTCTTCTATCGGA
PB-005	*Tif3*_ΔRRM-C	CCAAGAAGAGGGGGAGGTGC
PB-006	*Tif3*_ΔNTDΔRRM-FW	CCACCATATGCCAAGAAGAGGGGGAGGTGCAGAT
PB-007	*Tif3*_Δr1-7-FW	GGTATTTTTGGTTTGTTGAGGAGCCGCAACAGAAACTGAAAC
PB-008	*Tif3*_Δr1-7-RV	CCTCAACAAACCAAAAATA
PB-009	*Tif3*_r1-7-FW	GTAG CCACCATATGCCAAGAAGAGGG
PB-010	*Tif3*_r1-7-RV	GTCAGCGGATCCTTATTACTTACCAAACTG
PB-011	*Tif3*-FW	GGCATATGATGGCTCCACCAAAGAAAACCG
PB-012	*Tif3*-RV	CGGATCCCTATTTCTTACCAACAACTTCCC
PB-013	*Tif3*-CFW	CCAAGGCTCTTGCAGACCACC
PB-014	*Tif3*-CRV	GGTGGTCTGCAAGAGCCTTGG

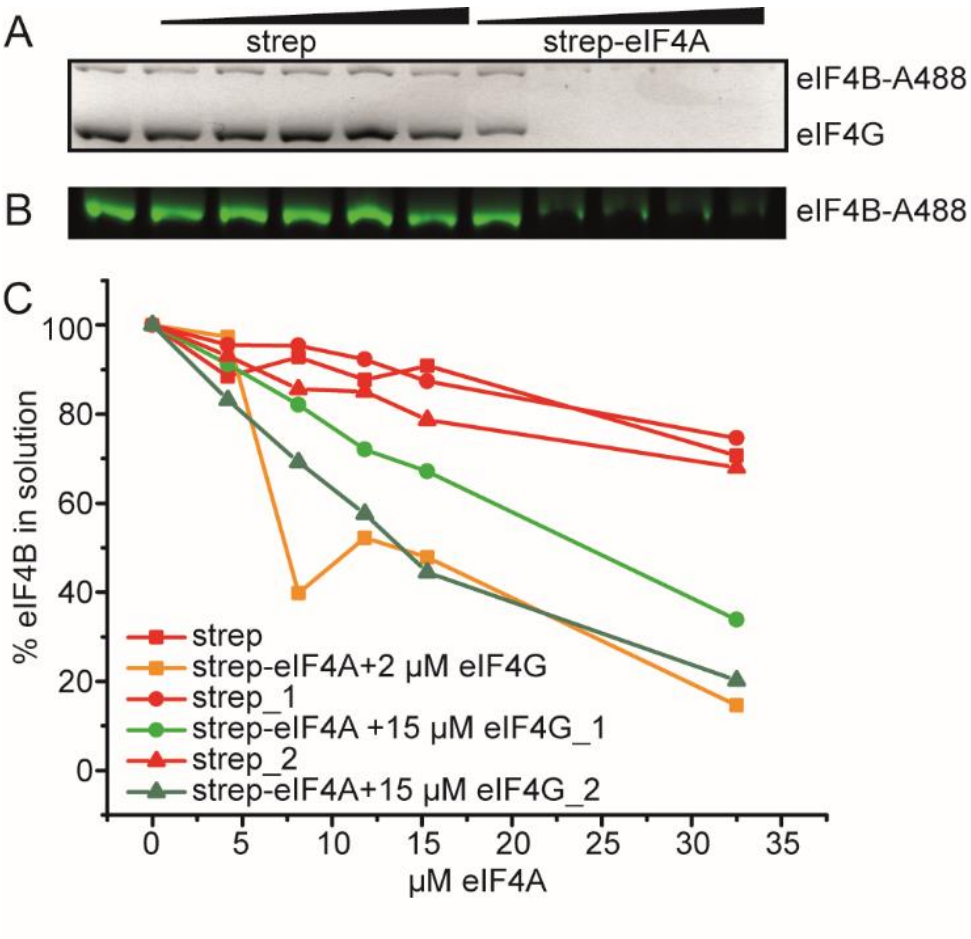
Supplementary Figures

Figure S1: **eIF4B binds to eIF4A in the presence of ADPNP and RNA.**



Supernatant depletion assay to follow binding of 0.5 µM eIF4B to eIF4A-bio in the presence of 5 µM RNA (32mer) and 10 mM ADPNP (Coomassie Blue staining). eIF4A-bio concentrations are 0, 4, 8, 12, 15 and 33 µM. eIF4B in the supernatant was quantified by densitometry. Red: depletion upon addition of streptavidin beads (negative control), yellow: depletion upon addition of eIF4A-conjugated streptavidin beads.

Figure S2: **eIF4B binding to eIF4A is not affected by eIF4G.**

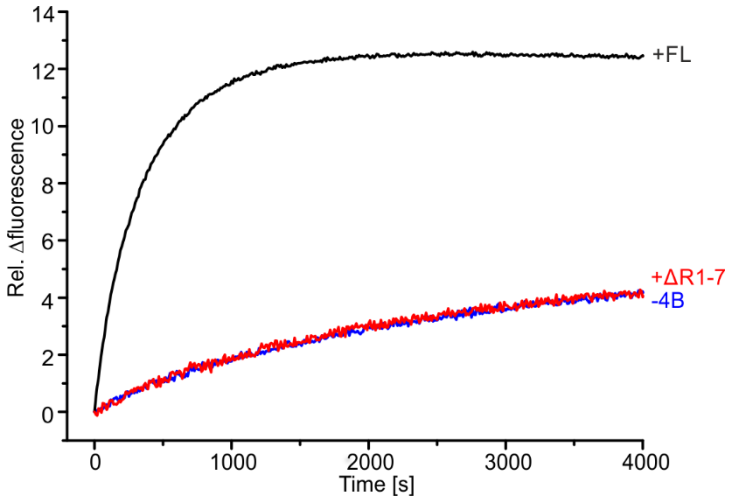


A: Supernatant depletion assay to follow binding of 0.25 µM eIF4B-A488 to eIF4A-bio in the presence of 10 mM ADPNP, 5 µM RNA (32mer) and 2 µM eIF4G (Coomassie Blue staining)

B: Same as in panel A, but with fluorescence detection.

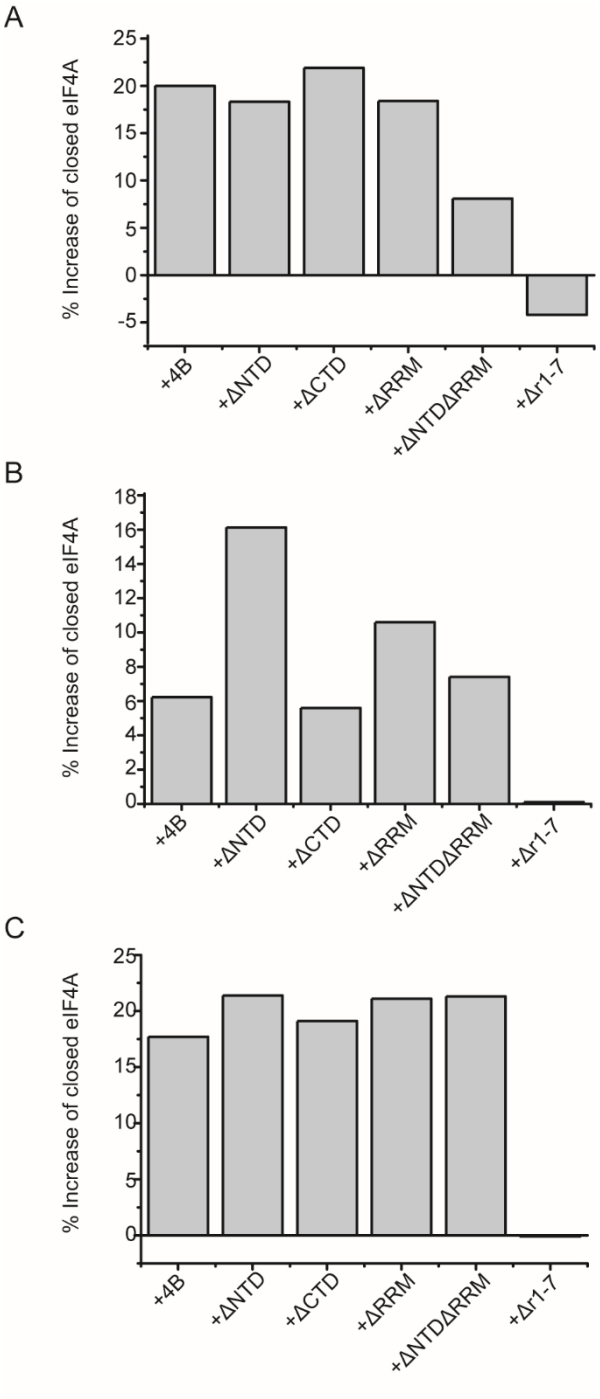
C: Quantification of the amount of eIF4B in the supernatant by fluorescence detection upon addition of streptavidin beads (red) or eIF4A-conjugated streptavidin beads in the presence of 2 µM (orange) or 15 µM eIF4G (light/dark green, two independent experiments).

Figure S3: **RNA unwinding by eIF4A in the presence of DNA trap.**



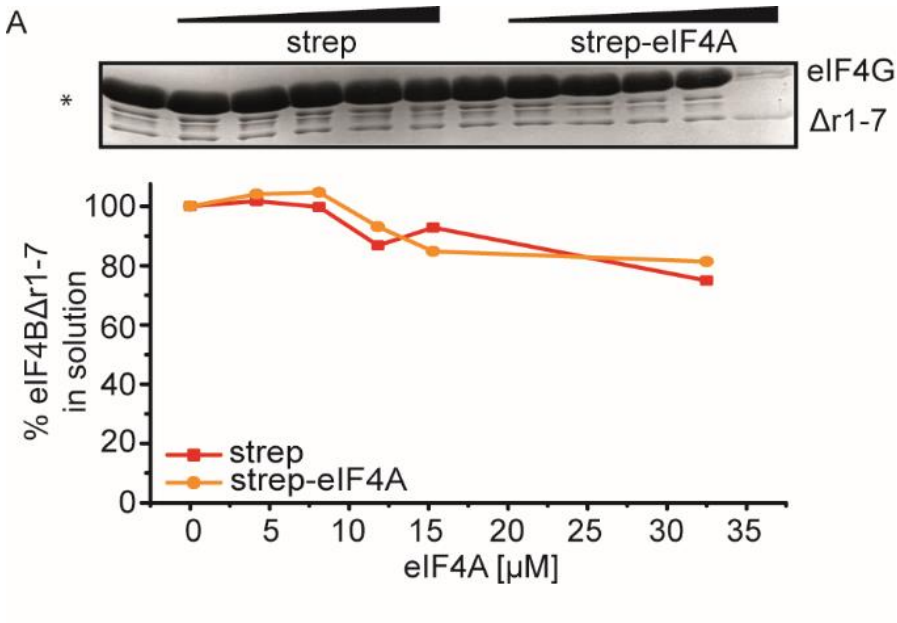
32mer/9mer RNA unwinding by eIF4A in the presence of eIF4G (blue), eIF4G and eIF4B (black) or eIF4G and eIF4B_Δr1-7 (red), followed by a decrease in FRET (i.e. decrease in Cy5 fluorescence, plotted here as an increase). The 9mer RNA strand released upon unwinding was trapped by a 9mer DNA oligonucleotide (5'-AGGTCCCAA-3'). Rate constants for unwinding are k_{unwind} = 0.42 10^{-3} s^{-1} (eIF4A/eIF4G), k_{unwind} = 2.67 10^{-3} s^{-1} (eIF4A/eIF4G/eIF4B) and k_{unwind} = 0.47 10^{-3} s^{-1} (eIF4A/eIF4G/eIF4B_Δr1-7).

Figure S4: **eIF4B_Δr1-7 fails to stimulate the eIF4A conformational change to the closed state.**



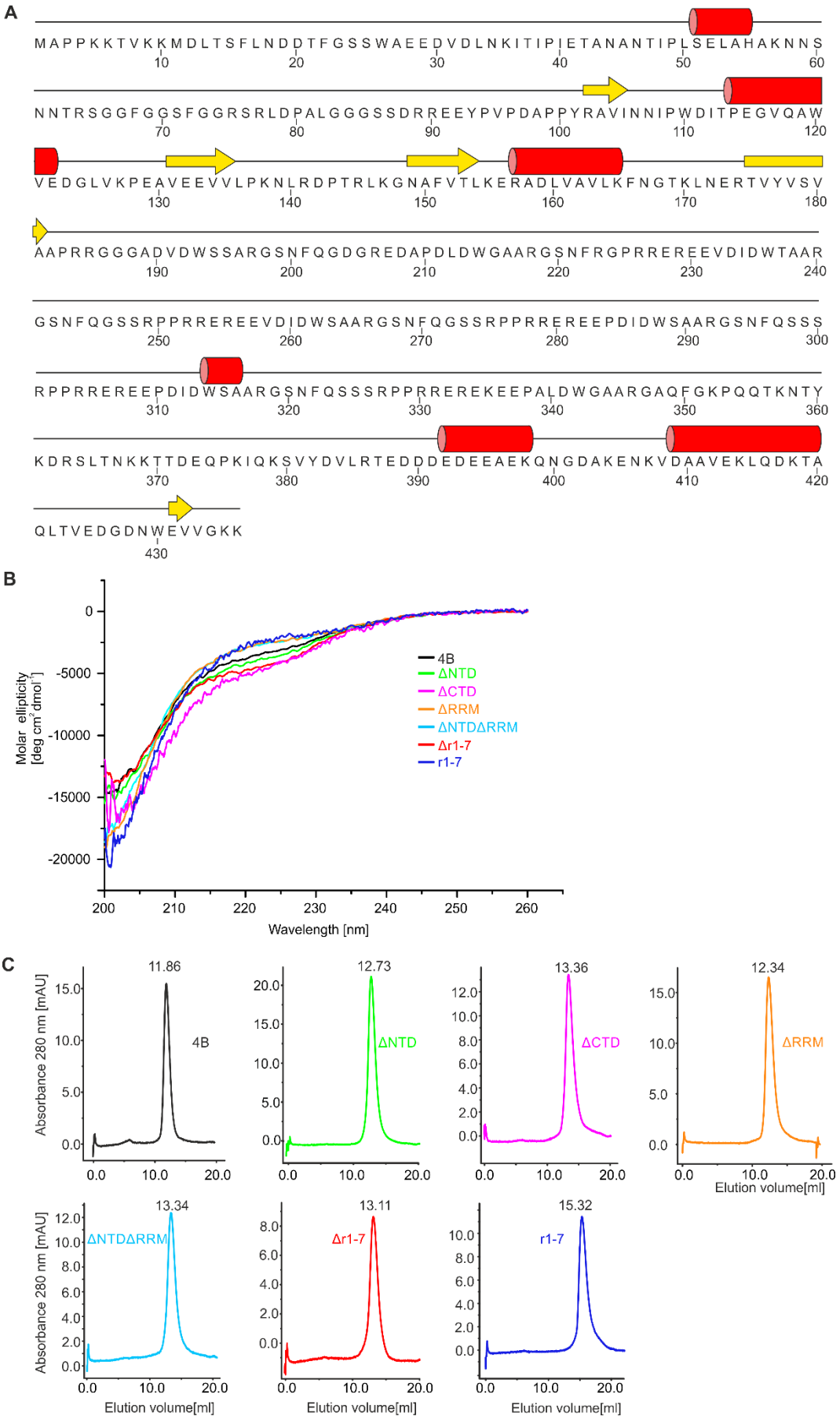
Increase of the population of eIF4A in the closed state upon addition of wild-type eIF4B and deletion variants. The three panels A, B, and C show the results from three independent experiments, performed with different batches of labeled eIF4A on different days. eIF4B lacking the 7-repeats region (eIF4B_Δr1-7) does not promote the eIF4A conformational change.

Figure S5: **eIF4G does not promote binding of eIF4B_Δr1-7 to eIF4A.**



Supernatant depletion assay with eIF4A-bio and 0.5 µM of eIF4B_Δr1-7 in the presence of 5 µM 32mer RNA, 15 µM eIF4G and 10 mM ADPNP (Coomassie Blue staining). eIF4B_Δr1-7 in the supernatant was quantified by densitometry. Red: depletion upon addition of streptavidin beads (negative control), yellow: depletion upon addition of eIF4A-conjugated streptavidin beads. The bands between the band for eIF4G and eIF4B_Δr1-7 are eIF4G degradation products.

Figure S6: **Analysis of folding of eIF4B variants.**

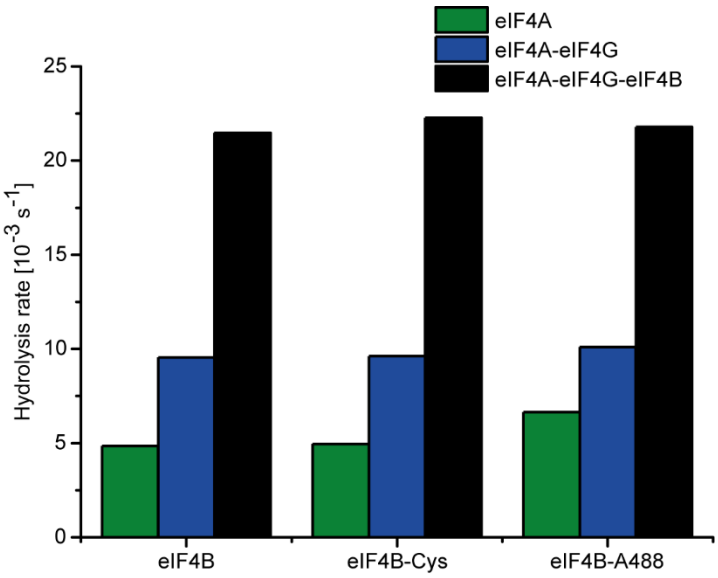


A: Prediction of secondary structure elements of eIF4B using PSIPRED {Jones, 1999 #3765}. β-strands are indicated by arrows, α-helical regions are depicted by cylinders.

B: Far-UV CD spectra of eIF4B variants. Measurements were performed with a protein concentration of 0.25 mg/ml in 10 mM HEPES/KOH, pH 7.5, 100 mM NaCl at 25 °C.

C: Elution profiles of eIF4B variants from an analytical Superdex 200 (10/300) chromatography column, equilibrated with 50 mM Tris/HCl, pH 7.5, 100 mM NaCl. Size-exclusion chromatography was performed with 250 µl of a ~3 µM solution of protein at room temperature with a flow rate of 0.7 ml min^{-1}.

Figure S7: **Fluorescently labeled eIF4B stimulates eIF4A ATPase activity**



eIF4B, eIF4B_248C/274C (eIF4B-Cys) and Alexa488-labeled eIF4B_248C/274C (eIF4B_A488) stimulate the RNA-dependent ATPase activity of eIF4A to the same extent. Experiments were performed in presence of 25 µM poly(U) RNA. In the absence of additional factors, eIF4A hydrolyzes ATP with k_{cat} = 4.9 10^{-3} s^{-1}, k_{cat} = 4.9 10^{-3} s^{-1} and k_{cat} = 6.6 10^{-3} s^{-1} (green) In the presence of eIF4G, the rates were k_{cat} = 9.6 10^{-3} s^{-1}, k_{cat} = 9.6 10^{-3} s^{-1}, and k_{cat} = 10.1 10^{-3} s^{-1}, respectively (blue). After addition of eIF4B, eIF4B-Cys or eIF4B-A488, k_{cat} values were increased to 21.5 10^{-3} s^{-1}, 22.3 10^{-3} s^{-1} and 21.8 10^{-3} s^{-1}, respectively (black).