Supplementary information

A conserved motif in human BTG1 and BTG2 proteins mediates interaction with the poly(A) binding protein PABPC1 to stimulate mRNA deadenylation

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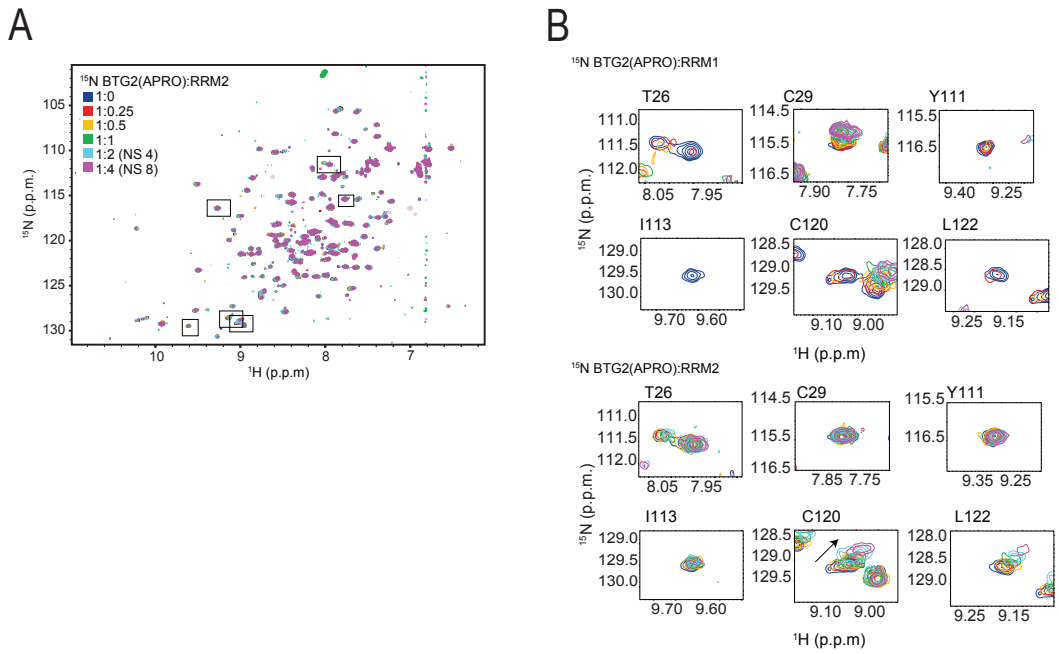
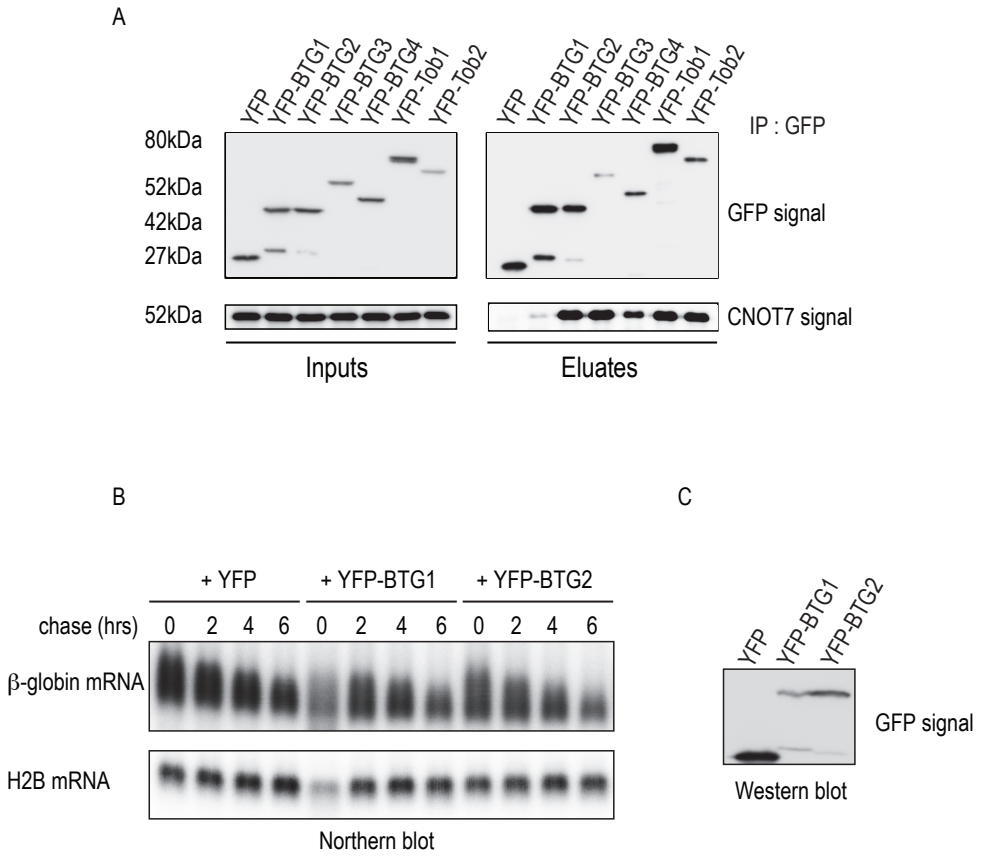
SUPPLEMENTARY FIGURES



Supplemental Figure 1 - Absence or weak interaction between BTG2 and PABPC1 RRM2

A) Overlay of ^{1}H-^{15}N HSQC spectra of free BTG2(APRO) (blue) and in complex with increasing amounts of PABP RRM2 (color code as in Fig. 1A). The number of scans (NS) is 2, otherwise indicated if different.

B) Comparison of key residues of RRM1 and RRM2.



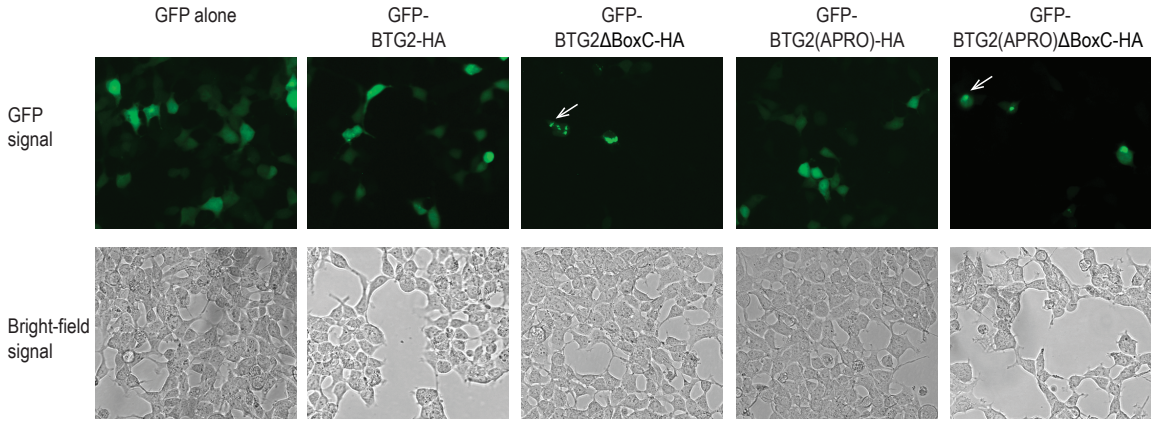
Supplemental Figure 2 - Expression of YFP-BTG1 stimulates mRNA decay despite a less efficient co-precipitation of endogenous CNOT7

A) Co-immunoprecipitation of endogenous CNOT7 with YFP-tagged BTG/Tob proteins. HEK293 cells were transfected with plasmids expressing YFP-BTG/Tob fusion proteins or only YFP as control. YFP-tagged proteins were precipitated with GFP-Trap magnetic agarose beads and the co-precipitation of endogenous CNOT7 (home-made polyclonal rabbit antiserum) was analyzed by western blotting.

B) Transcriptional chase experiments showing mRNA decay of the β-globin mRNA reporter in the presence of ectopically expressed YFP proteins. HEK293-TOF cells were co-transfected with reporter plasmid and plasmids expressing YFP-BTG1, YFP-BTG2 or YFP alone as control. Chase time indications correspond to hours after doxycyclin addition. Experiments were repeated twice. Signals obtained for replication-dependent H2B mRNAs

that are not poly-adenylated are shown as loading controls. Quantification of the results indicated half-lives of 12.25 ± 2.15, 7.7 ± 0.31 or 7.7 ± 1.53 hours respectively for the reporter co-transfected with YFP, or YFP-BTG1 or YFP-BTG2.

C) A western blot analysis of the cell lysates corresponding to the transfections performed in B is shown as a control.



Supplemental Figure 3 - GFP-BTG2 derivatives deleted of the boxC motif accumulate in cellular aggregates

HEK293 cells were transfected with plasmids expressing GFP-BTG2 fusion derivatives or GFP alone as indicated. 24 hours after transfection, images of living cells were captured with a FLoid Cell Imaging Station (Life Technologies). Arrows point to some aggregates.

SUPPLEMENTARY TABLES

Supplementary Table 1: Plasmids used in this study

Plasmid	Vector	Insert	Reference/construction
pBS2446		cDNA human BTG2 IMAGE 5186556	
pBS2561	pET24 derivative	Encodes 6His-mouse CNOT7	Reference 1
pBS2800	pTet-ß-globin		Reference 1
pBS3352	pGEX-2T	Encodes GST-BTG2(APRO) (amino acids 1 to 126)	Reference 1
pBS3415	pET24 derivative	Encodes 6His-human PABPC1(FL)	Reference 1
pBS3425		human Tob1 cDNA	Open Biosystems clone MHS1010-7430248
pBS3426		human BTG3 cDNA	Open Biosystems clone LIFESEQ4640478
pBS3428		human BTG1 cDNA	Open Biosystems clone MHS1010-9206137
pBS3499	pCIneo	Encodes human Tob1 APRO domain (amino acids 1 to 117) with C-terminal HA tag	Reference 1
pBS3503	pGEX-2T	Encodes GST-Tob1(APRO) (amino acids 1 to 117)	Reference 1
pBS4406	pP6	Encodes a Gal4-Activating-Domain – PABPC1 (amino acids 1 to 146) fusion protein	Reference 1
pBS4409	pP6	Encodes a Gal4-Activating – Domain – human CNOT7 fusion protein	Reference 1
pBS4413	pB27	Encodes a LexA-Binding-Domain - BTG2(APRO) fusion protein	Reference 1
pBS4550	pB27	Encodes a LexA-Binding-Domain – BTG1(APRO) fusion protein	Reference 1
pBS4551	pB27	Encodes a LexA-Binding-Domain – Tob1(APRO) fusion protein	Reference 1
pBS4611	pET24 derivative	Encodes 6His-PABPC1(1-190)	Reference 1
pBS4849	pCIneo	Encodes Tob1(APRO)DGSICVLYvdd-HA	Site-directed mutagenesis of pBS3499 with OBS5628 and OBS5629 to change sequence KGPVK (amino acids 106 to 110) in Tob1 to DGSIC
pBS4944	pET24 derivative	Encodes 6HIS-PABPC1-RRM2 (amino acids 85 to 190)	PCR on pBS3415 with OBS5453+OBS6068 cut XbaI-BamHI + pET24 cut XbaI-BamHI
pBS5189	pEGFP-C3	Encodes GFP-BTG2(APRO)-HA	Reference 1
pBS5193	pEGFP-C3	Encodes GFP-BTG2(APRO)kGpvkVLYEEA-HA	Site-directed mutagenesis of pBS5189 with OBS5449 and OBS5450 to change sequence DGSIC (amino acids 116 to 120) in BTG2 to KGPVK
pBS5194	pEGFP-C3	Encodes GFP-Tob1(APRO)-HA	Reference 1
pBS5295	pEGFP-C3	Encodes GFP-BTG2-HA	Reference 1
pBS5335		human BTG4 cDNA	Dharmacon clone ID 5271706
pBS5525	pcDNA6.0	Encodes V5-His-humanTob2	This work

pBS5581	pcDNA3-YFP	Encodes YFP	
pBS5582	pcDNA3-YFP	Encodes YFP-humanBTG1(FL)	PCR on pBS3428 with G3668 and G3669 cut BglII-XhoI + pBS5581 cut BamHI-XhoI
pBS5583	pcDNA3-YFP	Encodes YFP-humanBTG3(FL)	PCR on pBS3426 with G3670 and G3671 cut EcoRI-XhoI + pBS5581 cut EcoRI-XhoI
pBS5584	pcDNA3-YFP	Encodes YFP-humanTob1(FL)	PCR on pBS3425 with G3674 and G3675 cut BglII-XhoI + pBS5581 cut BamHI-XhoI
pBS5585	pcDNA3-YFP	Encodes YFP-humanBTG2(FL)	PCR on pBS5295 with G2711 and G2752 cut BamHI-XhoI + pBS5581 cut BamHI-XhoI
pBS5586	pcDNA3-YFP	Encodes YFP-humanBTG4(FL)	PCR on pBS5335 with OBS7390+OBS7391 cut BsaI-XhoI + pBS5581 cut BamHI-XhoI
pBS5594	pcDNA3-YFP	Encodes YFP-humanTob2(FL)	PCR on pBS5525 with OBS7392+OBS7393 cut BamHI-XhoI + pBS5581 cut BamHI-XhoI
pBS5799	pCIneo	Encodes Tob1(APRO)DGSICVLYEEA-HA	Site-directed mutagenesis of pBS4849 with OBS_VDDEEA_F and OBS_VDDEEA_R to change sequence VDD (amino acids 114 to 116) in Tob1 to EEA
pBS5875	pEGFP-C3	Encodes GFP-Tob1(APRO) DGSICVLYvdd-HA	PCR cut XhoI-PstI amplified with OBS3338 and OBS6169 from pBS4849
pBS5876	pEGFP-C3	Encodes GFP-Tob1(APRO) DGSICVLYEEA-HA	PCR cut XhoI-PstI amplified with OBS3338 and OBS6169 from pBS5799
pBS5895	pB27	Encodes a LexA-Binding-Domain – Tob1(APRO) DGSICVLYvdd fusion protein	PCR cut SfiI amplified with OBS5268 and OBS8262 from pBS5875
pBS5896	pB27	Encodes a LexA-Binding-Domain – Tob1(APRO) DGSICVCLYEEA fusion protein	PCR cut SfiI amplified with OBS5268 and OBS8261 from pBS5876
pBS5903	pEGFP-C3	Encodes GFP-BTG2(APRO)DboxC	PCR cut XhoI-EcoRI amplified with OBS_deltaboxc_F and OBS_deltaboxc_R from pBS5189
pBS5915	pEGFP-C3	Encodes GFP-BTG2DboxC-HA	Site-directed mutagenesis of pBS5295 with OBS_btg2FLdeltaboxC_F and OBS_btg2FLdeltaboxC_R to delete sequence DGSICVLYEEA (amino acids 116 to 126) in BTG2
pBS5923	pEGFP-C3	Encodes GFP-BTG2(APRO) kGpvkVLYvdd-HA	Site-directed mutagenesis of pBS5189 with OBS_btg2KGPVKVLYVDD_F and OBS_btg2KGPVKVLYVDD_R to change sequence DGSICVLYEEA (amino acids 116 to 126) in BTG2 to KGPVKVLYVDD
pBS5973	pGEX-2T	Encodes GST-Tob1(APRO) DGSICVLYEEA	Site-directed mutagenesis of pBS3503 with OBS8569 and OBS8570 to to change sequence KGPVKVLYVDD to DGSICVLYEEA
pBS6111	pEGFP-C3	Encodes GFP-BTG2(APRO)knnafivaEEA-HA	Site-directed mutagenesis of pBS5189 with OBS8573 and OBS8574 to change sequence DGSICVLY (amino acids 116 to 123) in BTG2 to KNNAFIVA
pBS6163	pETGB1-1a		kind gift from G. Stier and A. Geerlof (EMBL, Heidelberg, Germany)

pBS6164	pETGB1-1a	Encodes 6His-GB1-PABPC1-RRM1 (amino acids 1 to 99)	PCR on pBS3415 with OBS1957+OBS6158 cut XhoI-BsaI + pBS6163 cut XhoI-NcoI
pBS6165	pETGB1-1a	Encodes 6His-GB1-BTG2(APRO)	PCR on pBS5295 with OBS668+OBS6726 cut EcoRI-BspHI + pBS6163 cut NcoI-EcoRI
pBS6166	pETGB1-1a	Encodes 6His-GB1-BTG2(APRO)kGpvkVLYEEA	PCR on pBS5193 with OBS668+OBS7487 cut EcoRI-BspHI + pBS6163 cut NcoI-EcoRI
pBS6167	pETGB1-1a	Encodes 6His-GB1-BTG2	PCR on pBS2446 with OBS668+OBS2316 cut EcoRI-BspHI + pBS6163 cut NcoI-EcoRI

Supplementary Table 2: Oligonucleotides used in this study

Name	**Sequence**
G2711	ATTGGATCCATGAGCCACGGGAAGG
G2752	GGCCTCGAGGCTGGAGACTGCCATCAC
G3668	ATATAGATCTATGCATCCCTTCTACACC
G3669	ATATCTCGAGTTAACCTGATACAGTCAT
G3670	ATATGAATTCATGAAGAATGAAATTGCT
G3671	ATATCTCGAGTTAGTGAGGTGCTAACAT
G3674	ATATAGATCTATGCAGCTTGAAATCCAA
G3675	ATATCTCGAGTTAGTTAGCCATAACAGG
OBS_btg2FLdeltaboxC_F	GCGGCCAGTGGGCCTCCCCAATGC
OBS_btg2FLdeltaboxC_R	GCATTGGGGAGGCCCACTGGCCGC
OBS_btg2KGPVKVLYVDD_F	CAAGGTCTTGTACGTGGACGACAATATGTACCCATAACGAC
OBS_btg2KGPVKVLYVDD_R	GTCGTATGGGTACATATTGTCGTCCCAACGTACAAGACCTTGA
OBS_deltaboxc_F	CCGCTCGAGACCATGAGCCACGGGAAGGGAA
OBS_deltaboxc_R	TATGAATTCTTACTCCCCAATGCGGTAGGA
OBS_VDDEEA_F	GGATCCATCTGCGTGCTTTACGAGGAGGCGAATTCCATGTACCC
OBS_VDDEEA_R	CCTAGGTAGACGCACGAAATGCTCCTCCGCTTAAGGTAGATGGG
OBS668	GGATGCGGCCGCAATCATGAGCCACGGGAAGGGAACCGA
OBS1957	GCGCTCATGAACCCCAGTGCCCCCAG

OBS2316	CGGGAATTCTAGCTGGAGACTGCCATCAC
OBS3338	GGCCTCGAGGGATCCATGCAGCTTGAAATCCAAGTAG
OBS5268	ATCGGCCGGACGGGCCATGCAGCTTGAAATCCAAGTAG
OBS5449	CCGCATTGGGGAGAAGGGCCCCGTCAAGGTCTTGTACGAGGAGGC
OBS5450	GCCTCCTCGTACAAGACCTTGACGGGGCCCTTCTCCCCAATGCGG
OBS5453	GCCGGATCCTTAGAATTCTTTTGCCCTAGCTCCAAGTTC
OBS5628	CCAAATTGGTGAAGACGGATCCATCTGCGTGCTTTACGTGGATG
OBS5629	CATCCACGTAAAGCACGCAGATGGATCCGTCTTCACCAATTTGG
OBS6068	GGCTCTAGATAAGGAGGATATATATGCATCACCATCACCATCACATGTGGTCTCAGCGTGATCCA
OBS6158	GCCCTCGAGTTAGCCTACTCCACTTTTGCGAAG
OBS6169	GACCTGCAGTCATGCGTAGTCTGGTACGTCGTAT
OBS6726	ACCGAATTCCTAGGCCTCCTCGTACAAGACGC
OBS7381	CTGGGATCCTTATGGCGACTGTCGAACC
OBS7390	AACGGTCTCGGATCCATGAGAGATGAAATTGCAACAA
OBS7392	TCAGGATCCATGCAGCTAGAGATCAAAGTGGC
OBS7393	AACCTCGAGTCAGTTGGCCAGCACCACGG
OBS7487	ACCGAATTCCTAGGCCTCCTCGTACAAGACCTT
OBS8261	ATCGGCCCCAGTGGCCCTTAATTCGCCTCCTCGTAAAGCACG
OBS8262	ATCGGCCCCAGTGGCCCTTAATTATCATCCACGTAAAGCACG
OBS8569	GTTTCTTACCAAATTGGTGAAGACGGCTCCATCTGCGTCTTGTACGAGGAGGCCAATTAGAATTCATCGTGACTG
OBS8570	CAGTCACGATGAATTCTAATTGGCCTCCTCGTACAAGACGCAGATGGAGCCGTCTTCACCAATTTGGTAAGAAAC
OBS8573	TCCTACCGCATTGGGGAGAAAAACAATGCATTCATTGTTGCC

	GAGGAGGCCAACATGTA
OBS8574	TACATGTTGGCCTCCTCGGCAACAATGAATGCATTGTTTTTCT CCCCAATGCGGTAGGA

SUPPLEMENTARY METHODS

Transcriptional chase experiments and Northern blot analysis

HEK293 Tet-Off cells were transfected with 0.4 μg of the pTet-β-globin plasmid (pBS2800) and 0.4 μg of the GFP-BTG expressing plasmids in 6-cm diameter culture dishes with Effectene transfection reagent (Qiagen). Two days after transfection, a transcriptional chase was performed by addition of doxycyclin (2 μg/ml). Chase times correspond to hours after doxycyclin addition and to times of RNA extraction.

10 μg of total RNA was electrophoresed onto 1.4% agarose/6% formaldehyde gels and transferred to Hybond-N+ membranes (GE Healthcare). After transfer, blots were stained with methylene blue to check for equal loading and hybridized to probes synthesized by in vitro transcription with the T7 RNA polymerase (Promega). Hybridization signals were visualized with Typhoon FLA 9500 (GE Healthcare).

SUPPLEMENTARY REFERENCES

1. Stupfler B, Birck C, Seraphin B, Mauxion F. BTG2 bridges PABPC1 RNA-binding domains and CAF1 deadenylase to control cell proliferation. Nature communications 2016; 7:10811.