Supplementary Information

Frequent loss of BTG1 activity and impaired interactions with the Caf1 subunit of the Ccr4-Not deadenylase in non-Hodgkin lymphoma

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Supplementary Materials and methods

Plasmid DNA preparation

For yeast two-hybrid analysis, full length BTG1 cDNA was amplified using Phusion DNA polymerase and primers 5'-AAAAAAGAATTCATGCATCCCTTCTACACCCGGGCC-3' and 5'-AAAAAAGTCGACTTAACCTGATACAGTCATCATATTG-3'. The product was cloned (EcoRI/SalI) into a modified pGAL4-BD.Cam plasmid (Agilent) containing an HA-epitope tag between the Gal4 DNA binding domain and the fusion protein. Plasmids containing BTG1 variants were generated following a modified Quikchange protocol (Agilent). The deletion mutation ΔN10 BTG1 was made using an alternative 5' oligonucleotide (5'-AAAAGAATTCATGGATAGGCGATA-3'). Plasmids pAD-Gal4-CNOT7 and pAD-Gal4-CNOT8 were described previously [1].

For the proliferation assay, variant BTG1 cDNAs were amplified using Phusion DNA polymerase (New England Biolabs) and sub-cloned into eukaryotic expression plasmid pCMV5-HA (EcoRI/SalI).

For the 3' UTR tethering assay, HA-BTG1 cDNAs were amplified using primers 5'-CCACTGCTGGGCCTGGACAGCACCCTCGAGTACCCATACGATGTTCCAG-3' and 5'-GGTCGACTCTAGAGGTACCACGCGTGAATTTTAACCTGATACAGTCATC-3' and inserted into the XhoI site of plasmid pCI-λN [2,3] using the NEBuilder HiFi DNA Assembly Cloning Kit (New England Biolabs) in a total volume of 3µl. The reporter plasmid pRL-5BoxB was described before [4].

All BTG1 cDNA sequences were confirmed by DNA sequencing (Source Bioscience).

Reverse transcriptase-quantitative PCR

Reverse transcriptase-quantitative PCR was used to measure the level of reporter mRNA. Total RNA was isolated 24 hours post transfection using the Macherey-Nagel RNeasy kit. QuantiTect Reverse Transcriptase (Qiagen) was used for the synthesis of cDNA free of plasmid contamination (300 ng total RNA template, 10 µl final reaction volume). In parallel, reactions were carried out in the absence of reverse transcriptase to verify the absence of plasmid contamination. Quantitative PCR was performed using an Applied Biosystems 7500 FAST PCR instrument. Reactions (10 µl) were assembled using the TaqMan Advanced Master Mix (Applied Biosystems) and primers specific for the gene tested (RT-Luciferase FW: 5'-TCGTCCATGCTGAGAGTGTC-3', RT-Luciferase RV: 5'-CTAACCTCGCCCTTCTCCTT-3') and a custom TaqMan probe (5'-FAM-AGTTCGCTGCCTACCTGGA-3'-NFQ-MGB). GAPDH (assay Hs02758991-g1, ThermoFisher Scientific) was used for normalisation. Reactions were incubated for 2 min at 50˚C, 10 min at 92˚C, followed by 40 cycles each consisting of 1 sec at 97˚C and 20 sec at 62˚C. The change in luciferase reporter mRNA levels was determined using the $2^{-\Delta\Delta Ct'}$ method [5].

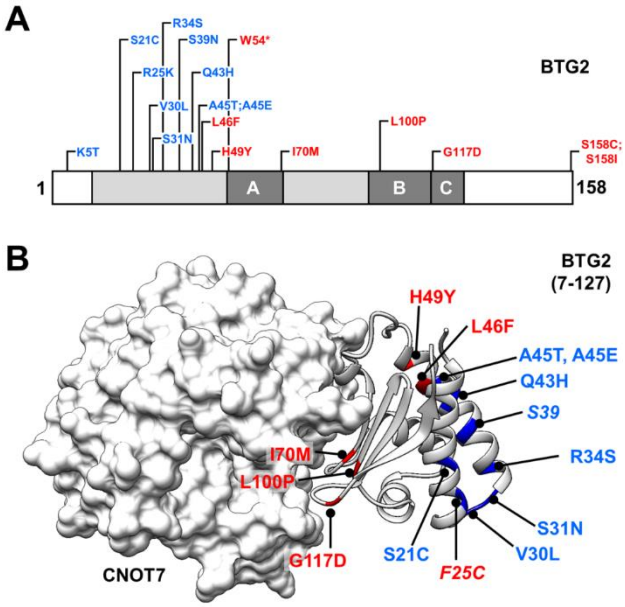
Supplementary Results

Supplementary Table 1. List of BTG2 mutations evaluated using the SIFT algorithm in different types of lymphoma.

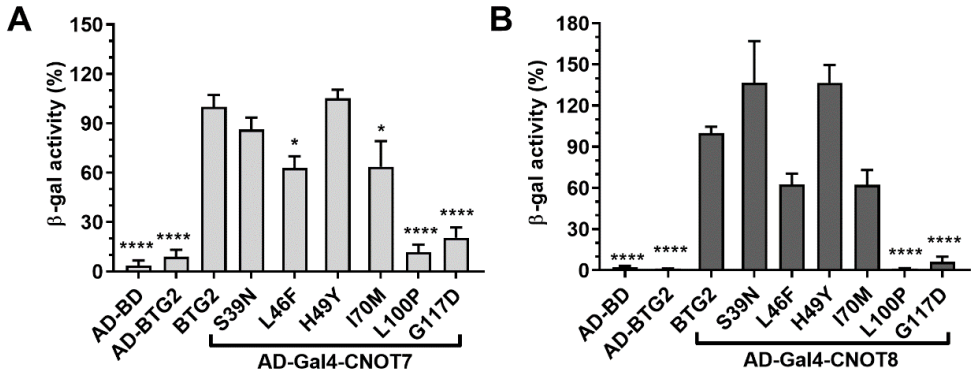
No	Position	Amino acid substitution	Mutation CDS	Codons	p	Prediction	Lymphoma sub-type[a]	Ref.
1	5	K → T	A>C	AGG>CGG	0.168	Tolerated	GCB	[6]
2	21	S → C	C>G	TCC>TGC	0.156	Tolerated	GCB	[6]
3	25	R → K	G>A	AGG>AAG	0.209	Tolerated	FL	[6]
4	30	V → L	G>T	GTG>TTG	1	Tolerated	ABC	[6]
5	31	S → N	G>A	AGC>AAC	0.48	Tolerated	GCB	[6]
6	34	R → S	G>C	AGG>AGC	0.077	Tolerated	ABC	[6]
7	36	K → Q	A>G	AAG>CAG	1	Tolerated	Burkitt's	[7]
8	39	S → N	G>A	AGC>AAC	0.156	Tolerated	GCB	[6]
9	43	Q → H	G>C	CAG>CAC	0.052	Tolerated	ABC	[6]
10	45	A → E	C>A	GCA> GAA	0.218	Tolerated	GCB	[6]
11	45	A → T	G>A	GCA> ACA	0.287	Tolerated	FL	[6]
12	46	L → F	C>T	CTC>TTC	0.009	Damaging	GCB	[6]
13	49	H → Y	C>T	CAC>TAC	0.003	Damaging	GCB	[6]
14	70	I → M	C>G	ATC>ATG	0.054	Damaging	Burkitt's	[7]
15	100	L → P	T>C	CTG>CCG	0	Damaging	Burkitt's	[7]
16	117	G → D	G>A	GGC>GAC	0.002	Damaging	Unknown	[6]
17	158	S → C	A>T	AGC>TGC	0	Damaging	Burkitt's	[7]
18	158	S → I	G>T	AGC>ATC	0	Damaging	Burkitt's	[7]

[a]Follicular Follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), germinal centre B-cell (GCB), activated B-cell (ABC) and multiple myeloma (MM). SIFT prediction software v 1.03 was used [8]. The zygosity was not identified in all BTG2 mutations.

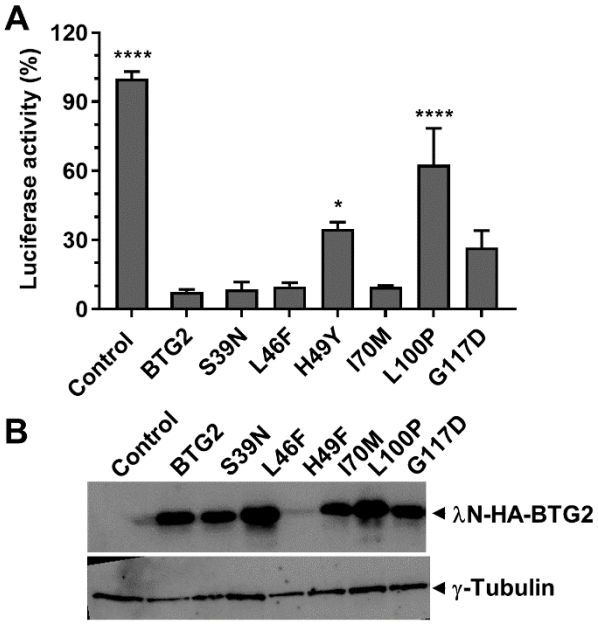
Supplementary Figures



Supplementary Figure 1. *In silico* analysis of lymphoma-associated BTG2 variants.
(A) Overview of lymphoma-associated mutations in BTG2. Mutations predicted to be damaging by the SIFT algorithm [8] are shown in red ($p < 0.05$); mutations in blue are predicted to be tolerated. The conserved BTG domain is shown in light grey; conserved regions Box A, B and C are shown in dark grey. **(B)** Structural mapping of BTG2 variants. Variants were mapped onto the structure of BTG2 [9]. The BTG2 structure (cartoon representation) was then superimposed on the BTG domain of TOB1 in complex with CNOT7 (surface representation) [10].



Supplementary Figure 2. Impaired interactions between lymphoma-associated BTG2 variants and the CNOT7 and CNOT8 deadenylase subunits of the Ccr4-Not complex. **(A)** Yeast two-hybrid interactions between CNOT7 and BTG2 variants. **(B)** Yeast two-hybrid interactions between CNOT8 and BTG2 variants. Error bars indicate the standard error of the mean (n = 3). P values were calculated using a one-way Anova and Dunnett's post-hoc test. * $p<0.05$, **** $p<0.0001$.



Supplementary Figure 3. Lymphoma-associated amino acid substitutions of BTG2 do not generally interfere with degradation of a tethered reporter mRNA.
(A) Luciferase reporter mRNA levels after expression of wild type BTG2 and lymphoma-associated BTG2 variants. HEK293T cells were co-transfected with a reporter plasmid containing a *Renilla* luciferase expression cassette containing five box B sequences in the 3′ UTR and a plasmid expressing λN-HA-BTG1 fusion proteins. Levels of the luciferase reporter mRNA were determined by reverse transcriptase-quantitative PCR. Error bars indicate the standard error of the mean (n = 3). P values were calculated by a one-way ANOVA with Tukey's post-hoc test. * $P < 0.05$, *** $p < 0.001$ and **** $p < 0.0001$ (compared to the cells expressing wild type λN-HA-BTG1). **(B) L**ysates of transfected cells were separated by SDS-PAGE and immunoblots probed with anti-HA antibodies to detect the λN-HA-BTG1 variants. Antibodies recognising γ-tubulin were used as loading controls.

Supplementary References

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