

Supporting Information

Table S1. Functional annotation of the BPLF1-interacting proteins involved in translation and RQC

Protein	MW [kDa]	Σ Coverage	Unique Peptides	Σ PSMs	Functional annotation
EIF2S1	36,1	15,6	4	28	Eukaryotic translation initiation factor 2 subunit 1. Functions in the early steps of protein synthesis by forming a ternary complex with GTP and initiator tRNA. The complex binds to a 40S ribosomal subunit followed by mRNA binding to form the 43S pre-initiation complex. Is a key component of the integrated stress response (ISR). Phosphorylation by metabolic-stress sensing protein kinases (EIF2AK1/HRI, EIF2AK2/PKR, EIF2AK3/PERK and EIF2AK4/GCN2) converts EIF2S1/eIF-2-alpha in a global protein synthesis inhibitor, leading to attenuation of cap-dependent translation and preferential translation of ISR-specific mRNAs, such as the transcriptional activators ATF4.
EIF3E2	52,2	11,5	4	8	Eukaryotic translation initiation factor 4E type 2. Binds the 7-methylguanosine-containing mRNA cap during the early steps of translation initiation acting as a repressor. In contrast to EIF4E, it is unable to bind eIF4G, suggesting that it may acts by blocking assembly of the IF4F complex.
EIF4G1	154,7	7,8	8	23	Eukaryotic translation initiation factor 4 gamma 1; Component of the eIF4F complex involved in the recognition of the mRNA cap. Participates in the ATP-dependent unwinding of 5'-terminal secondary structure and recruitment of mRNA to the ribosome.
EIF4G2	98,1	4,1	3	5	Eukaryotic translation initiation factor 4 gamma 2. May play a role in the switch from cap-dependent to IRES-mediated translation during mitosis, apoptosis, and viral infection. Cleaved by caspases and viral proteases.
EIF5A	16,1	19,7	3	18	Eukaryotic translation initiation factor 5A; mRNA-binding protein involved in translation elongation. Function in mRNA turnover, probably acting downstream of de-capping. Involved in actin dynamics and cell cycle progression, mRNA decay and maintenance of cell wall integrity.

KHSRP	73,0	8,6	5	9	Far upstream element-binding protein 2. Part of a ternary complex that binds to the downstream control sequence (DCS) of the pre-mRNA. Mediates exon inclusion in transcripts that are subject to tissue-specific alternative splicing. May interact with single-stranded DNA from the far-upstream element (FUSE). Also involved in the degradation of mRNAs that contain AU-rich elements (AREs) in their 3'-UTR.
RPLP2	11,7	38,3	3	10	60S ribosomal protein L2, mitochondrial
RPL8	22,4	22,4	3	9	60S ribosomal protein L8
RPL9	20,9	36,8	6	35	60S ribosomal proteins 9, belongs to the L6P family of ribosomal proteins.
RPL13	18,8	20,1	5	43	60S ribosomal protein L13
RPL13A	23,6	20,7	4	39	60S ribosomal protein L13a. Associated with ribosomes but is not required for canonical ribosome function. Upon interferon-gamma induced phosphorylation dissociates from the ribosome and assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of inflammatory mRNAs.
RPL15	20,5	19,5	3	27	60S ribosomal protein L15
RPL17	19,6	34,3	5	40	60S ribosomal protein L17. Belongs to the ribosomal protein uL22 family
RPL18A	16,7	33,3	5	32	60S ribosomal protein L18A. Belongs to the ribosomal protein eL20 family.
RPL26	11,6	19,6	4	28	60S ribosomal protein 26. Belongs to the ribosomal protein L24P family Is located near the docking sites for the translocation machinery signal recognition particle (SRP), the SEC61 translocon, and the oligosyl transferase (OST) complex.
RPL30	12,6	35,1	3	17	60S ribosomal protein L30
RPL34	13,3	16,2	3	4	60S ribosomal protein L34
RPL36	12,2	21,9	3	14	60S ribosomal protein L36
RPL38	8,2	50,0	4	24	Ribosomal protein L38; Belongs to the ribosomal protein eL38 family
RPS11	18,4	32,3	4	10	Ribosomal protein S11; Belongs to the ribosomal protein uS17 family
RPS19	16,1	23,5	5	38	40S ribosomal protein S19
RPS5	14,8	17,2	3	18	40S ribosomal protein S5, small ribosomal subunit uS7

RPS7	22,1	25,8	5	27	40S ribosomal protein S7; Required for rRNA maturation; Belongs to the ribosomal protein eS7 family
RPN1	68,5	26,5	12	35	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1; Essential subunit of the N-oligosaccharyl transferase (OST) complex that catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains. The complex binds to ribosome to take part in co-translational protein folding. Following UFMylation of the N-terminal cytoplasmic domain, RPN1 delivers a stress ER stress signal during peptide translocation, which can ultimately induce ER-phagy or UPR. The protein forms part of the regulatory subunit of the 26S proteasome and may mediate binding of ubiquitin-like domains to the proteasome.
SEC63	87,9	7,6	4	11	Translocation protein 63 homolog; ER membrane protein, member of the Sec61 complex that mediates co-translational the transport of polypeptides across the ER. Cooperates with SEC62 to facilitate targeting to into the SEC61 channel-forming translocon. The complex may also perform retrograde transport of ER proteins that are subject to the ubiquitin-proteasome-dependent degradation.
SRP68	66,1	10,5	6	19	Signal recognition particle (SRP) subunit 68. The SRP targets secretory proteins to the rough ER. SRP68 binds the 7S RNA, which recruits SRP72 to the complex. The ribonucleoprotein complex may interact with docking proteins in the ER membrane and possibly participate in elongation arrest.
SRPRA	66,5	7,2	4	20	Signal recognition particle receptor subunit alpha. Component of the SRP receptor complex. In conjunction with the SRP complex ensures the correct targeting of the nascent secretory proteins to the ER membrane. Forms a guanosine 5'-triphosphate (GTP)-dependent complex with the SRP subunit SRP54.
SRPRB	29,7	46,1	9	23	Signal recognition particle receptor subunit beta; Component of the SRPR Has GTPase activity and may mediate the membrane association of SRPR.
UFL1	82,0	8,9	4	7	UFM1 E3 ligase. Is recruited to the ER membrane by DDRGK1 and mediates UFMylation of RPN1 and RPL26/uL24, thereby promoting ER-phagy, which inhibits the unfolded protein response (UPR). DDRGK1 and CDK5RAP3 serve as substrate adapters for UFMylation and ER-phagy. Catalyzes UFMylation of various subunits of the ribosomal complex or associated components, such as RPS3/uS3, RPS20/uS10, RPL10/uL16, RPL26/uL24 and EIF6.

Table S2. Reagents used in this paper

Reagent	Source	Identifier
Antibodies		
Mouse monoclonal anti- β -actin clone AC-15	Sigma-Aldrich	Cat# A5441, RRID: AB_476744
Mouse monoclonal anti-GAPDH	Millipore	Cat#CB1001
Mouse monoclonal anti-tubulin	Millipore	Cat#CP06
Mouse monoclonal anti-FLAG	Sigma-Aldrich	Cat# F3165, RRID: AB_259529)
Rabbit polyclone anti-FLAG	Sigma-Aldrich	Cat#F7425, RRID: AB_439687
Anti-FLAG M2 affinity gel	Sigma-Aldrich	Cat#A2220 RRID:AB_10063035
Rabbit monoclonal anti-Myc Tag	Cell signaling technology	Cat#2278
Mouse monoclonal anti-S Tag,	Millipore	Cat#71549-3 RRID: AB_11210600
Mouse anti-Myc Tag agarose (4A6)	Millipore	Cat# 16-219
S-Protein Agarose	Novagen	Cat#69704
protein-G coupled Sepharose beads	GE Healthcare	Cat#17-0885-01
GammaBind Plus Sepharose	Cytiva	Cat#17088601
Rabbit IgG isotype control	Abcam	Cat# ab172730 RRID:AB_2687931
Donkey anti-Mouse IgG (H+L), Alexa Fluor 555	Thermo Fisher Scientific	Cat# A-31570, RRID: AB_2536180
Donkey Anti-Rabbit IgG (H+L) Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21206, RRID: AB_2535792
Goat Anti-Rabbit IgG (H+L) Antibody, Alexa Fluor 647	Thermo Fisher Scientific	Cat#A21245 RRID: AB_141775
Rabbit polyclonal anti-ZNF598	Invitrogen	Cat# PA559777 RRID: AB_2650226
Rabbit polyclonal anti-LTN1	GeneTex	Cat# GTX18154
Rabbit polyclonal anti-SEC63	Bethyl Laboratories	Cat# A305-084A, RRID: AB_2631479
Rabbit polyclonal anti-RPN1	Bethyl Laboratories	Cat# A305-026A, RRID: AB_2621220
Mouse monoclonal anti-RPN1	Santa Cruz Biotechnology	Cat# sc-48367, RRID: AB_628221
Rabbit polyclonal anti-SRPRB	Bethyl Laboratories	Cat# A305-440A, RRID: AB_2631831
Rabbit polyclonal anti-SRP68	Bethyl Laboratories	Cat# A303-955A, RRID: AB_2620304
Mouse monoclonal anti-VCP	Abcam	Cat# ab11433 RRID:AB_298039
Rabbit monoclonal anti-RPS10	Abcam	Cat# ab151550 RRID:AB_2714147
Rabbit monoclonal anti-RPS3	Abcam	Cat# ab128995 RRID:AB_11145466

Rabbit polyclonal anti-SEC61B	Thermo Fisher Scientific	Cat# PA3-015 RRID: AB_2239072
Rabbit polyclonal anti-eIF4G2 (DAP5)	Bethyl Laboratories	Cat# A302-249A, RRID: AB_1730977
Mouse monoclonal anti-EIF5A	BD Biosciences	Cat# 611976 RRID:AB_399398
Mouse monoclonal anti-eIF4G1 (2A9)	Abnova	Cat# H00001981-M10, RRID: AB_606171
Rabbit anti-Phospho-eIF2 α -Ser51 (D9G8) XP	Cell Signaling Technology	Cat# 3398, RRID: AB_2096481
Mouse monoclonal anti-eIF2 α (L57A5)	Cell Signaling Technology	Cat#2103, RRID: AB_836874
Rabbit monoclonal anti-ATF4 (D4B8)	Cell Signaling Technology	Cat# 11815, RRID: AB_2616025
Mouse monoclonal anti-CHOP (L63F7)	Cell Signaling Technology	Cat# 2895, RRID: AB_2089254
Mouse monoclonal anti-Puromycin (12D10)	Millipore	Cat# MABE343 RRID: AB_2566826
Rat monoclonal anti-EBV-BPLF1	MAB core facility, Helmholtz Center, Munich, Germany ¹	N/A
Rat monoclonal anti-EBV-EBNA1	MAB core facility, Helmholtz Center, Munich, Germany	N/A
Rabbit polyclonal anti-EBV-BdRF1	Dr. Jaap M. Middelorp, VU University Medical Center, Amsterdam, NL	N/A
Rabbit polyclonal anti-EBV-BGLF1	Dr. Jaap M. Middelorp, VU University Medical Center, Amsterdam, NL	N/A
Mouse monoclonal anti LMP1 (CS1-4)	Dako, Glostrup, Denmark	M0897
Rabbit polyclonal anti-EBV BXLF1	ASLA Biotech, Riga Latvia	N/A
Mouse monoclonal anti-EBV-BMRF1	Dr. Jaap M. Middelorp, VU University Medical Center, Amsterdam, NL	N/A
Chemicals, peptides, and recombinant proteins		
IGEPAL CA-630	Sigma-Aldrich	I3021; CAS: 9002-93-1
MgCl ₂	Merck	Cat# M1028
Sodium dodecyl sulphate	Sigma-Aldrich	L3771; CAS:151-21-3
N-Ethylmaleimide	Sigma-Aldrich	E1271; CAS:128-53-0
Iodoacetamide	Sigma-Aldrich	I1149; CAS:144-48-9
Sodium deoxycholate monohydrate	Sigma-Aldrich	D5670; CAS:145224-92-6
Triton X-100	Sigma-Aldrich	T9284; CAS:9002-93-1

Bovine serum albumin	Sigma-Aldrich	A7906; CAS:9048-46-8
Tween-20	Sigma-Aldrich	P9416; CAS: 9005-64-5
Trizma base	Sigma-Aldrich	93349; CAS:77-86-1
Ciprofloxacin	Sigma-Aldrich	Cat#17850; CAS: 85721-33-1
Doxycycline cyclate	Sigma-Aldrich	D9891; CAS: 24390-14-5
MG132	Sigma-Aldrich	M7449; CAS: 200-664-3
Anisomycin	Sigma-Aldrich	A5862; CAS:22862-76-6
Cycloheximide	Sigma-Aldrich	C4859; CAS:66-81-9
Polybrene	Sigma-Aldrich	TR-1003-G;
Complete protease inhibitors cocktail	Roche Diagnostic	Cat#04693116001
Phosphatase inhibitor cocktail	Roche Diagnostic	Cat#04906837001
DAPI	Sigma-Aldrich	Cat#9542 CAS:28718-90-3
Mowiol	Calbiochem	Cat# 475904 CAS:9002-89-5
HA-Ubiquitin-VS	R&D systems	Cat# U-212-025
PERK inhibitor I, GSK2606414	Sigma-Aldrich	516535 CAS 1337531-89-1
GCN2 inhibitor (A92)	AXON Medchem	Axon 2720; CAS:1448693-69-3
PKR inhibitor	Sigma-Aldrich	Cat# 527451 CAS 608512-97-6
ISRIB	Sigma-Aldrich	SML0843 CAS:1597403-47-8
Kits		
DC Protein Assay quantification kit	Bio-Rad	Cat#A500-0116
SuperSignal™ WestPico PLUS Chemiluminescent Substrate	Thermo Scientific	Cat#XE356732
Lipofectamine 3000 transfection kit	Invitrogen	Cat#L3000-015
jetOPTIMUS DNA transfection reagent	Polyplus	Cat#101000006
Q5® Site-Directed Mutagenesis Kit	New England Biolabs	Cat#E0554S
NEBuilder® HiFi DNA Assembly Master Mix	New England Biolabs	Cat#E2621S
Quick-RNA MiniPrep kit	Zymo Research	Cat#R1055
RNA clean and concentrator	Zymo Research	Cat#R1013
SuperScript VILO cDNA Synthesis kit	Invitrogen	Cat#11754050
LC FS DNA Master SYBR Green I	Roche	Cat#12239264001
Experimental models: Cell lines		
RPMI1640	Sigma-Aldrich	Cat# R8758
DMEM	Sigma-Aldrich	Cat# D6429
EBSS	Gibco	Cat# 1854705
HeLa	ATCC	RR-B51S
HEK293T	ATCC	CRL3216
HEK293T-EBV- BPLF1wt/cm	Gupta et al. Plos Path, 2018 ²	

LCLwt/cm (Tet-on BZLF1)	Li et al. Plos Path, 2021 ³	N/A
Recombinant DNA		
eGFP-ER-K20-RFP	gift from Yihong Ye	Addgene # 133861
pmGFP-P2A-K0-P2A-RFP	gift from Ramanujan Hegde	Addgene # 105686
pmGFP-P2A-K(AAA)20-P2A-RFP	gift from Ramanujan Hegde	Addgene # 105688
pCMV10-3xFLAG-BPLF1	Gastaldello et al. Nat cell Biol, 2010 ⁴	N/A
pCMV10-3xFLAG-BPLF1 ^{C61A}	Gastaldello et al. Nat cell Biol, 2010 ⁴	N/A
pCMV10-3xFLAG-UL36	Gupta et al. Front Immunol., 2021 ⁵	N/A
pCMV10-3xFLAG-UL48	Gupta et al. Front Immunol., 2021 ⁵	N/A
pCMV10-3xFLAG-ORF63	Gupta et al. Front Immunol., 2021 ⁵	N/A

Table S3. PCR and qPCR primers

Gene	Forward primer(5'-3')	Reverse primer(5'-3')
MLN51 qPCR	CAAGGAAGGTCGTGCTGGTT	ACCAGACCGGCCACCAT
BPLF1 qPCR	CATACACCGTGCGAAAAGAA	GATGGCGGGTAATACATGCT
EBNA1 qPCR	GGCAGTGGACCTCAAAGAAG	CTATGTCTTGGCCCTGATCC
BMRF1 qPCR	TGACAAGCTTATGGAAACCACTCAGACTCTCC	TCGATGAATTCTTAAATGAGGGGGTTAAAGGCC
LytLMP1 qPCR	CAGCACAATTCCAAGGAACA	TTTCCCCAGTCACCCTCCT
BXLF1 qPCR	AGGCTGCCGGACATCAAT	TTCTGTGCACGAAGTTTTC
BPLF1 ^{C61A} mutagenesis	CGGCATCCAGGCAGTCAGCAAC TGC	GCAAAGCGGCCAAACCTTG
GFP-nonSTOP cloning	CGATCGACTAGTACGCGTGTTACAAATAAAGCAATAGCATCA	ACGCGTACTAGTCGATCGCTTGTACAGCTCGTCCATG

References

1. van Gent M, *et al.* Epstein-Barr virus large tegument protein BPLF1 contributes to innate immune evasion through interference with toll-like receptor signaling. *PLoS Pathog* **10**, e1003960 (2014).
2. Gupta S, Yla-Anttila P, Callegari S, Tsai MH, Delecluse HJ, Masucci MG. Herpesvirus deconjugases inhibit the IFN response by promoting TRIM25 autoubiquitination and functional inactivation of the RIG-I signalosome. *PLoS Pathog* **14**, e1006852 (2018).
3. Li J, *et al.* The Epstein-Barr virus deubiquitinating enzyme BPLF1 regulates the activity of topoisomerase II during productive infection. *PLoS Pathog* **17**, e1009954 (2021).
4. Gastaldello S, *et al.* A deneddylase encoded by Epstein-Barr virus promotes viral DNA replication by regulating the activity of cullin-RING ligases. *Nat Cell Biol* **12**, 351-361 (2010).
5. Gupta S, Yla-Anttila P, Sandalova T, Achour A, Masucci MG. Interaction With 14-3-3 Correlates With Inactivation of the RIG-I Signalosome by Herpesvirus Ubiquitin Deconjugases. *Front Immunol* **11**, 437 (2020).

Supplementary figures

Figure S1. Catalytically active BPLF1 promotes the translation of all classes of viral mRNAs during productive EBV infection. (A). Catalytically active BPLF1 promotes the expression of a selection of early and late viral proteins. Western blots of cell lysates from the induction experiments shown in (B) were probed with the indicated antibodies. (B). Densitometric quantification of protein and mRNA expression in two to four independent experiments. Due to undetectable expression in uninduced cells, protein levels are expressed as the ratio of band intensity in cells expressing wild-type and mutant BPLF1.

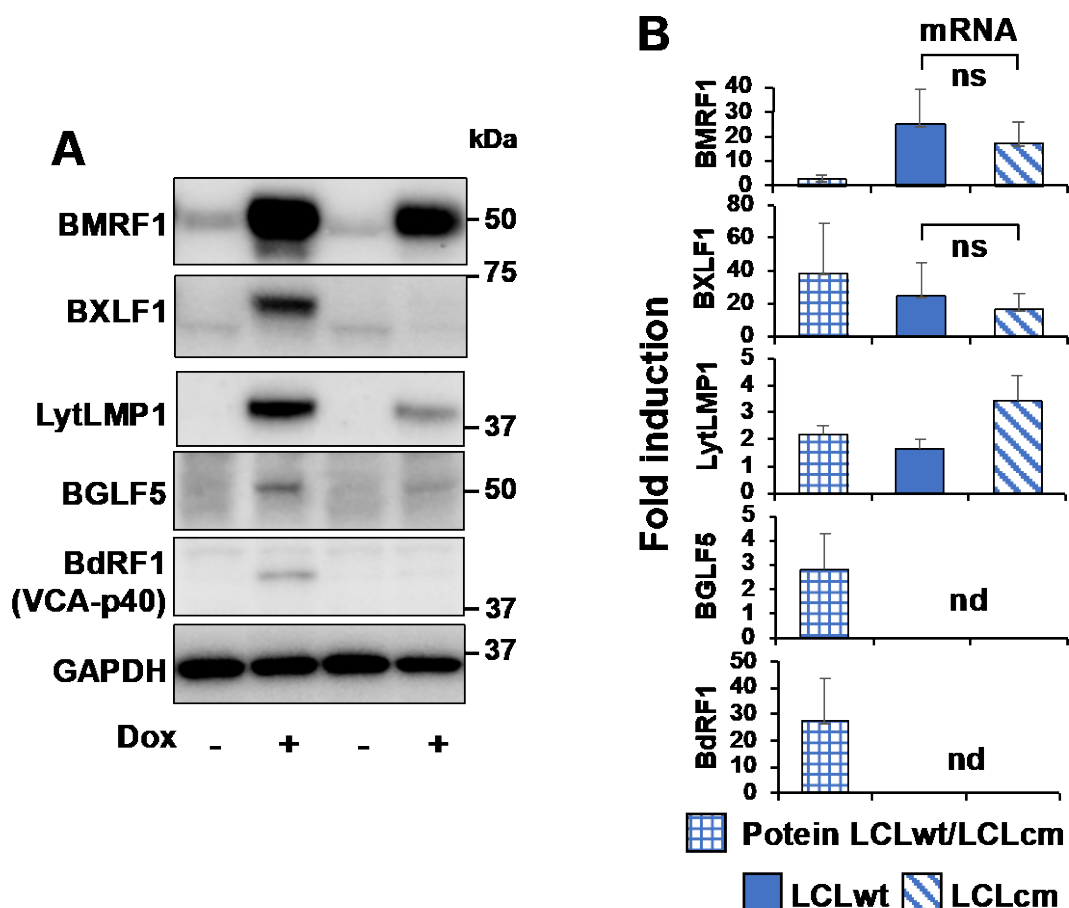


Figure S2. The N-terminal domains of the BPLF1 homolog encoded by HSV1, HCMV and KSHV are active vDUBs. Lysates of HeLa cells transfected for 24 h with the indicated plasmids were incubated with 1 μ g of the functional probe Ub-VS that forms covalent bonds with the catalytic Cys residue of active DUBs. After incubation for 1 h at 37°C the lysates were fractionated by SDS-PAGE and the viral proteins were detected in western blots probed with the anti-FLAG antibody. DUB activity was confirmed by a shift in molecular weight corresponding to the size of the covalently bound probe (indicated by arrows). The shift was not observed in lysates of cells expressing the catalytic mutant BPLF1^{C61A}.

