

Molecular Cell, Volume 57

## **Supplemental Information**

### **TopBP1 Interacts with BLM to Maintain Genome Stability but Is Dispensable for Preventing BLM Degradation**

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PROTEIN	PEPTIDES
TOPBP1	124
BLM	51
MDC1	49
RFC1	45
53BP1	25
RFC3	22
IWS1	22
TCOF1	21
RFC4	20

**Figure S1 (related to Figure 1): Identification of TopBP1-associated proteins by mass spectrometry.** Pulldowns were carried out from 293FT cells transiently transfected with plasmids expressing GFP-tagged TopBP1 or GFP as a control. The top hits found exclusively in the GFP-TopBP1 sample are shown in the table.

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Human      133 DTALKKLEFSSSPDSLSTI-NDWDDMDDFDTSETSKSFVTPPQSHFVRVSTAQKSKKGRNFFKAQLYTT
Mouse      133 VAVFKKLEFSSSA---DSL-SDWADMDDFDMSASDAF-ASLAKNPATRVSTAQKMKTKRNFPPRKA
Chicken    97  GSVSVSLPSSG--PAAYTG-DEWDDMDDFDLGFEKKFS-RPAVLSP-----KGPRT--
Lizard     125 QLENPKADHNSSYSIINIDVWDDIDDFEIAARQEKKCSKSPASLSS---NTLLAKKSKAPKNQPLTSE
Xenopus    122 DAASKKTGINTSFGSVTSL-EEDWDLDDFDTSVSPKSH-AGKGG-----KTPOK-C
Zebrafish  138 NDQTVELKHI---SQLSF-NEWDDLDDEFETPVKSRVAS-PVAGTST-----KKPVSVD

Human      NTV-----KTDLPPSSESEQIDLTEE-----QKDDS-EWLSSDVVICIDGPIAE
Mouse      NAV-----KTDLTPPSPECLQVDLTKESSEEEEE---EEEAEGA-DCLSRDVIICIDNDSASE
Chicken    -----PQGRGLRVSKARADVGPQEHGSE-VSCSQGSSQGLICLDGAGAPA
Lizard     QLVPLVSRQGSQQL---CNEGTVNKSNTIQIPDDDDQ-----EEN-APGQSVICLGPITS-H
Xenopus    K---NTSPVASFKIQSISPEGPTTEKHDCAKLLYDNNEVASEPRKNLHAKTA-ESPQSLVCLASVEP-T
Zebrafish  Q---NTSSSCSSK---CEETKVNESQA-----TET-ITAPKDVLSAANGVS-T

Human      VHINEDAQESDSL-KTH-----LEDERD---NSEKKNLLEEALHST
Mouse      ELTEKDTQESQSL-KAH-----LGAERG---DSEKKSHEDEAVFHSV
Chicken    AAVLSDNGMEEARAATN-----GGSGESQKLSNGEKSSQLEPGDV-GN
Lizard     DGSYEDVPANRPL-GSP-----PGSKKVESLEI-RNDKGEKPGSA-CN
Xenopus    NLERDMCRNTDYL-G-----TDDLE-HDQETL-----
Zebrafish  ETAEREPEDS-PIKSKRPPKSVQHTALLSDTEDEEIIHCVSPDTNQKNFKSVAVAEKDKWGPENVI-D-

Human      EKVPCIE-FDDDDYDTDFVPPSPEEIIISA-SSSSKCLSTLKD-----LDTSDRK--EDVLST-SKD
Mouse      QNTEYFE-HNDNDYDIDFVPPSPEEIIIST-ASSSLKCSMLKD-----LDDSDKE--KGLIST-SEE
Chicken    ELLADIE-LEED-DYLDVPPSPEEELPS-FSPSVNVSNIFK-----ESPTDGR--SAVHGTESEP
Lizard     DPEVCNE-SETE-DYVDFIIPSPSEEEAPSSASSFSISYIFK-----ESAVEKTRSPQALK--SSS
Xenopus    -SQVLI-EEDD-CEPDIIPSPSDESLS-SPPVLKVISQQRKHVSSSLTDVNDCENT--TDH-----
Zebrafish  -SDDCENNHYE-GFEDEIIPSPTEETS-LSVSDSEKSSSEP-----VTPANKK--ESS-----

Human      LLSKPEKMSMQEL---NPETSTDCDARQIS-LQQQLIHVMEHICKLIDTTPDDKLLDCCGNEILLQQRN
Mouse      LLSKPEEMTTHKS---DAGTSKCDQAQIR-IQQQLIHVMEHICKLVDTVPTDELEALNCGTEILLQQRN
Chicken    ELMAPKQPAEQD---SSAEHADKGLH-LEQQLYSVMEDICKLVDAIPLHELTSISCAKELLQQRQ
Lizard     GASSGRKAQEDSDEGGFSEVQCYLSGKFFP-SGQSLYSVMEDICKLVDSIPEPDLRFLSCGSELIRHRD
Xenopus    LQ--GQS-----VSTSLDS---K-VPSQLLTLMLICLDLVKTIPISELHVLSCGLQKKRDR
Zebrafish  RSASGLP-----APLDQSAKGLKGADDAIFSVMESICCLVDIPEHELIALTCGTEILLQRA

Human      IRRKLLTEVDF-NKSDASLLG-----SLWRYRFPDSIDGPMEGDSCPTG-----NSMK
Mouse      IRRKLLAEAGF-NGNDVRLLG-----SLWRHRPDSIDNTVQGDSCPVG-----HPNK
Chicken    LRRKLLADSGA-LNTN-----SNGPRNWKACVQDPPSRPGTPLCSGFGRGVS
Lizard     YRYDGLSDSGVFRLEE-----ENWDSPPGGMSTSTQ-----PSVR
Xenopus    MRKRLSNDVSV-FRSPADSSVSLTSTCTSSQNRDFNWNAPKGAESLSGS-----SVSK
Zebrafish  HRKRILAKGGS-SRTSH-----SDSISTPG-----FLN

Human      ELNFSHLP-----SNSVSPGDCLLTTT---LGRKGFSAIR-----KNLF-----
Mouse      ELNSPYLL-----SHSPSTEECLPTTT---PGRKGFSAIR-----KNLF-----
Chicken    SVG-----STPK---STNLPSVLSRT---VNSSSFSITIR-----NQTLDKLDTSYSS
Lizard     KFG-----VFERHHNFHSTPSVVPFV---QGTSNVSLSRFPF-----NSAL-----
Xenopus    VFKFNKLAVDIGTKESSENSANSAPNFMEKI---GNRTSFSFRA-GG-----DSIM-----
Zebrafish  RPTFGVTP-----SNLT---SLTPVTSKGREGVKIGFSFRK-SIASVMSVGDSESVF-----

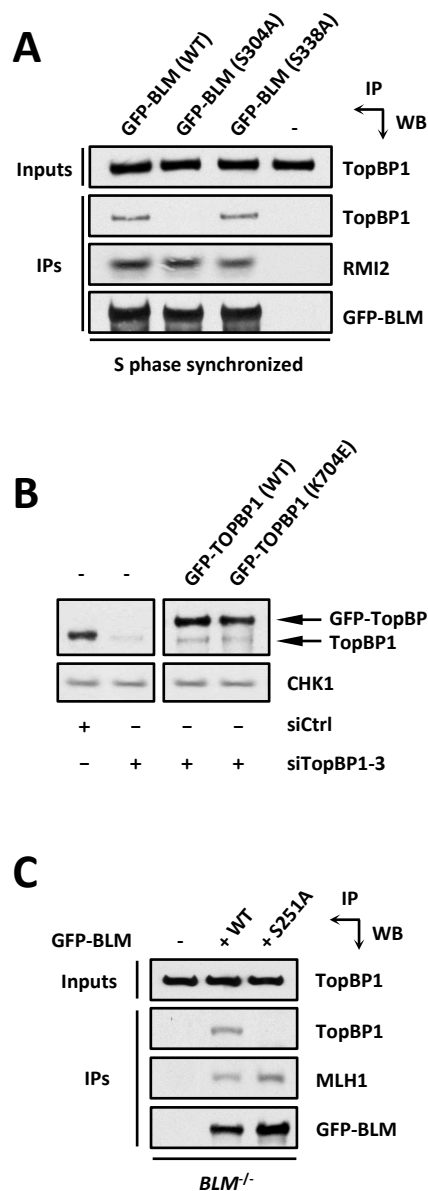
Human      ---ER-----PLFNTHLQKSFVSSNWAE--TP-----
Mouse      ---ER-----PLLNSHLQKSFVSSNWAE--TP-----
Chicken    KETDQEVICL-EPAALPSPKVNGKGSTLSRPPSEASF-----NGSWCE--KP-----
Lizard     ---EN-----GI-----SPL-----
Xenopus    ---EN-----SFNF-----HSSVL-----
Zebrafish  ---EDSDCIINGVETPGGTWPNPNSSTKISAG-RDTF-----NGSIQTLKPKESKTDKCYRSLFSN

Human      -RLGKKNESYFPGN--VLTSTAV----KDQNK-HTASIND----LERE---TQPSYDIDNEDIDDFD
Mouse      -RMENRNESTDFPGS--VLTSTTV----KAQSK-QAASGWN----VERH---GQASYDIDNENIDDFD
Chicken    -T-GRDSGNWRVPER--PTASTAL----KAQ---HTAPAGNPASGCWD-V---NDTDFLDHEDIDDFD
Lizard     ----QNASLSFKPKR--GSSGERMSENFMNPEMNVTAESDS----LA-N---RAGNEDDNEFDIDDLN
Xenopus    ----SNSRFNTPQNEKPISSSTC---TRP---YSQPIDD---MD-N---PDLDFDIDNEDIDDLN
Zebrafish  ES-SNQTDLFYSPKR--VDSGSRNAD---SSVEI-NIAGSSS----LR-TGAEPVDDFDLDDDEFIDDFD

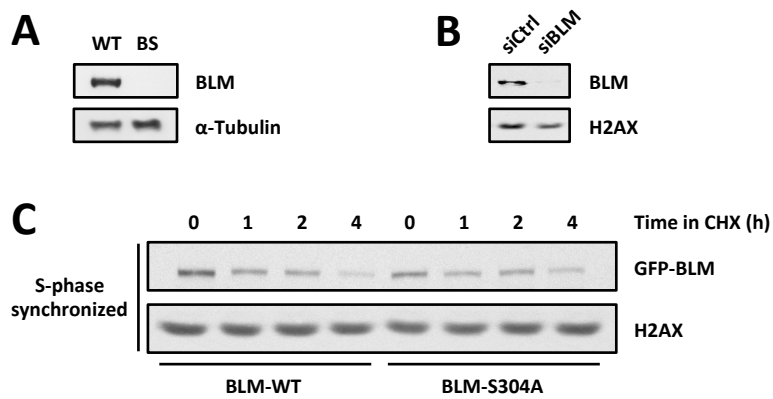
Human      DDD---DWEDIMHNLA-SSKSTAYQPI 587
Mouse      DDDDDDDWENIMHNFPA-SSKSTATYPI 595
Chicken    EGW----EEAVAPEAAPEAPPAPQWQPL 561
Lizard     ELD-----EIIQLSGA-EQPLQASCLPA 569
Xenopus    DIH---CLDSPAAPSV--SSKNVPQYPTI 552
Zebrafish  ENDIPDYEEPPSVLES-RNNSGVKTPSV 616

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**Figure S2 (related to Figure 2): Sequence alignment of BLM 133-587.** Alignments were carried out using T-Coffee (<http://tcoffee.org.cat/apps/tcoffee/do:regular>) and Boxshade ([http://www.ch.embnet.org/software/BOX\\_form.html](http://www.ch.embnet.org/software/BOX_form.html)) programs. Conserved residues are highlighted in black, similar residues are highlighted in grey. Human S304 and equivalent residues in other organisms are shown in bold and indicated with a red triangle.



**Figure S3 (related to Figure 3): Ser338 of BLM is not required for TopBP1 binding in S phase and generation of cells stably expressing point mutations of BLM and TopBP1.** (A) Mutation of BLM Ser338 to alanine does not affect its interaction with endogenous TopBP1 in S phase. Pulldowns were carried out from 293FT cells transiently transfected with the indicated plasmids and released from a thymidine block. (B) Complementation of TopBP1-depleted cells with WT and K704E TopBP1. U2OS cells stably expressing GFP-tagged WT or K704E TopBP1 and their parental line were transfected with siRNA targeting firefly luciferase (siCtrl) or the TopBP1 UTR (siTopBP1-3). CHK1 is a loading control. (C) Ser251 is the chicken equivalent of Ser304 in human BLM. *BLM*<sup>-/-</sup> DT40 cells were stably complemented with the indicated plasmids. MLH1 was used as a positive control for BLM binding as it binds the C-terminus of BLM.



**Figure S4 (related to Figure 4): Specificity of the BLM antibody used in this study and S phase stability of mutant BLM that cannot bind TopBP1. (A)** Western blot of cell extracts from a Bloom syndrome (BS) patient and a healthy donor (WT). Tubulin is a loading control. **(B)** Western blot of extracts from U2OS cells treated with siRNA targeting firefly luciferase (siCtrl) or BLM (siBLM). H2AX is a loading control. **(C)** BLM-TopBP1 interaction does not maintain BLM stability in S phase. U2OS cells stably expressing GFP-BLM proteins were released from a thymidine block, and cycloheximide (CHX) was added for the indicated times before harvesting for western blotting. H2AX is a loading control.

## Supplemental Experimental Procedures

### Primary antibodies used in this study.

Target	Antibody No.	Source	Application*	Dilution
BLM	A300-110A	Bethyl Laboratories	IP, WB	1:5000
BLM-pS304	-	Dr. Yi Wang	WB	1:500
CHK1	sc8408	Santa Cruz Biotechnology	WB	1:1000
CldU	ab6326	Abcam	IF	1:400
E1A	-	Dr. Roger Grand	WB	1:15
FANCI	4578	Cell Signaling Technology	WB	1:1000
FLAG	F1804	Sigma-Aldrich	WB	1:1000
GFP	11 814 460 001	Roche	WB	1:2000
GST	sc138	Santa Cruz Biotechnology	WB	1:2000
H2AX	ab11175	Abcam	WB	1:5000
IdU	347580	BD Biosciences	IF	1:25
Ku70	ab3114	Abcam	WB	1:400
Ku80	MS-285-P1	Thermo Scientific	WB	1:2000
MDC1	-	Dr. Grant Stewart	WB	1:1000
MLH1	ab92312	Abcam	WB	1:10,000
NBS1	ab7860	Abcam	WB	1:5000
p53	sc126	Santa Cruz Biotechnology	WB	1:1500
RMI1	ab70525	Abcam	WB	1:1000
RMI2	NBP1-89962	Novus Biologicals	WB	1:5000
TOP3A	14525-1-AP	Proteintech	WB	1:1000
TopBP1	A300-111A	Bethyl Laboratories	IP, WB	1:5000
$\alpha$ -Tubulin	T5168	Sigma-Aldrich	WB	1:100,000

\*IF, immunofluorescence; IP, immunoprecipitation; WB, Western blotting.

**Plasmids used in this study.**

<b>Plasmid expressing</b>	<b>Vector</b>	<b>Organism</b>	<b>Details</b>
GFP-BLM	pEGFP-C1	Human	(Hu et al., 2001)
GFP-BLM (133-1417)	pEGFP-C1	Human	(Hu et al., 2001)
GFP-BLM (1-1222)	pEGFP-C1	Human	T1223X mutation introduced by site-directed mutagenesis into GFP-BLM
GFP-BLM (1-587)	pEGFP-C1	Human	K588X mutation introduced by site-directed mutagenesis into GFP-BLM
GFP-BLM (1-133)	pEGFP-C1	Human	T134X mutation introduced by site-directed mutagenesis into GFP-BLM
GFP-BLM (S304A)	pEGFP-C1	Human	S304A mutation introduced by site-directed mutagenesis into GFP-BLM
GFP-BLM (S338A)	pEGFP-C1	Human	S338A mutation introduced by site-directed mutagenesis into GFP-BLM
GFP-BLM	pEGFP-C1	Chicken	(Hirano et al., 2005)
GFP-BLM (S251A)	pEGFP-C1	Chicken	S251A mutation introduced by site-directed mutagenesis into GFP-BLM
GFP-TopBP1	pIRESneo2	Human	(Cescutti et al., 2010)
GFP-TopBP1 (BRCT1-K154/5A)	pIRESneo2	Human	K154/5A mutation introduced by site-directed mutagenesis into GFP-TopBP1
GFP-TopBP1 (BRCT5-K704A)	pIRESneo2	Human	K704A mutation introduced by site-directed mutagenesis into GFP-TopBP1
GFP-TopBP1 (BRCT5-K704E)	pIRESneo2	Human	K704E mutation introduced by site-directed mutagenesis into GFP-TopBP1
GFP-TopBP1 (BRCT5-W711R)	pIRESneo2	Human	W711R mutation introduced by site-directed mutagenesis into GFP-TopBP1
GFP-TopBP1 (BRCT7-K1317A)	pIRESneo2	Human	K1317A mutation introduced by site-directed mutagenesis into GFP-TopBP1
GST-TopBP1 (BRCT4+5)	pGEX-4T-1	Human	(Schmidt et al., 2008)
GST-TopBP1 (BRCT5)	Contact Wang et al.	Human	(Wang et al., 2013)
SFB-BLM	Contact Wang et al.	Human	(Wang et al., 2013)
SFB-BLM (K3A)	Contact Wang et al.	Human	(Wang et al., 2013)
SFB-BLM (S304A)	Contact Wang et al.	Human	S304A mutation introduced by site-directed mutagenesis into SFB-BLM
SFB-BLM (S338A)	Contact Wang et al.	Human	(Wang et al., 2013)

**siRNAs used in this study.**

<b>Name</b>	<b>Target</b>	<b>Sequence</b>
siCtrl	Firefly luciferase	5'-CGUACGCGGAAUACUUCGA-3'
siTopBP1-1	TopBP1	5'-ACAAAUACAUGGCUGGUUA-3'
siTopBP1-2	TopBP1	5'-CUCACCUUAUUGCAGGAGA-3'
siTopBP1-3	TopBP1	5'-GUAAAUAUCUGAAGCUGUA-3'
siTopBP1-4	TopBP1	5'-GCACAAGGUUAAUGAGGA-3'
siBLM	BLM	5'-GCUAGGAGUCUGCGUGCGA-3'

**Peptides used in this study.**

<b>Peptide</b>	<b>Sequence</b>
S304	Biotin-DTDFVPPSPEEII-NH <sub>2</sub>
pS304	Biotin-DTDFVPP[pS]PEEII-NH <sub>2</sub>
S338	Biotin-CKEDVLSTSKDLLSKPE-NH <sub>2</sub>
pS338	Biotin-CKEDVLST[pS]KDLLSKPE-NH <sub>2</sub>

Peptides were found to be >95% pure after reverse phase high-performance liquid chromatography and compositions were verified by mass spectrometry.



## Supplemental References

Hirano, S., Yamamoto, K., Ishiai, M., Yamazoe, M., Seki, M., Matsushita, N., Ohzeki, M., Yamashita, Y.M., Arakawa, H., Buerstedde, J.M., *et al.* (2005). Functional relationships of FANCC to homologous recombination, translesion synthesis, and BLM. *The EMBO journal* 24, 418-427.

Schmidt, U., Wollmann, Y., Franke, C., Grosse, F., Saluz, H.P., and Hanel, F. (2008). Characterization of the interaction between the human DNA topoisomerase IIbeta-binding protein 1 (TopBP1) and the cell division cycle 45 (Cdc45) protein. *The Biochemical journal* 409, 169-177.