Fig. S1a

BLM

TR(TOP3A/RMI1-binding domain)

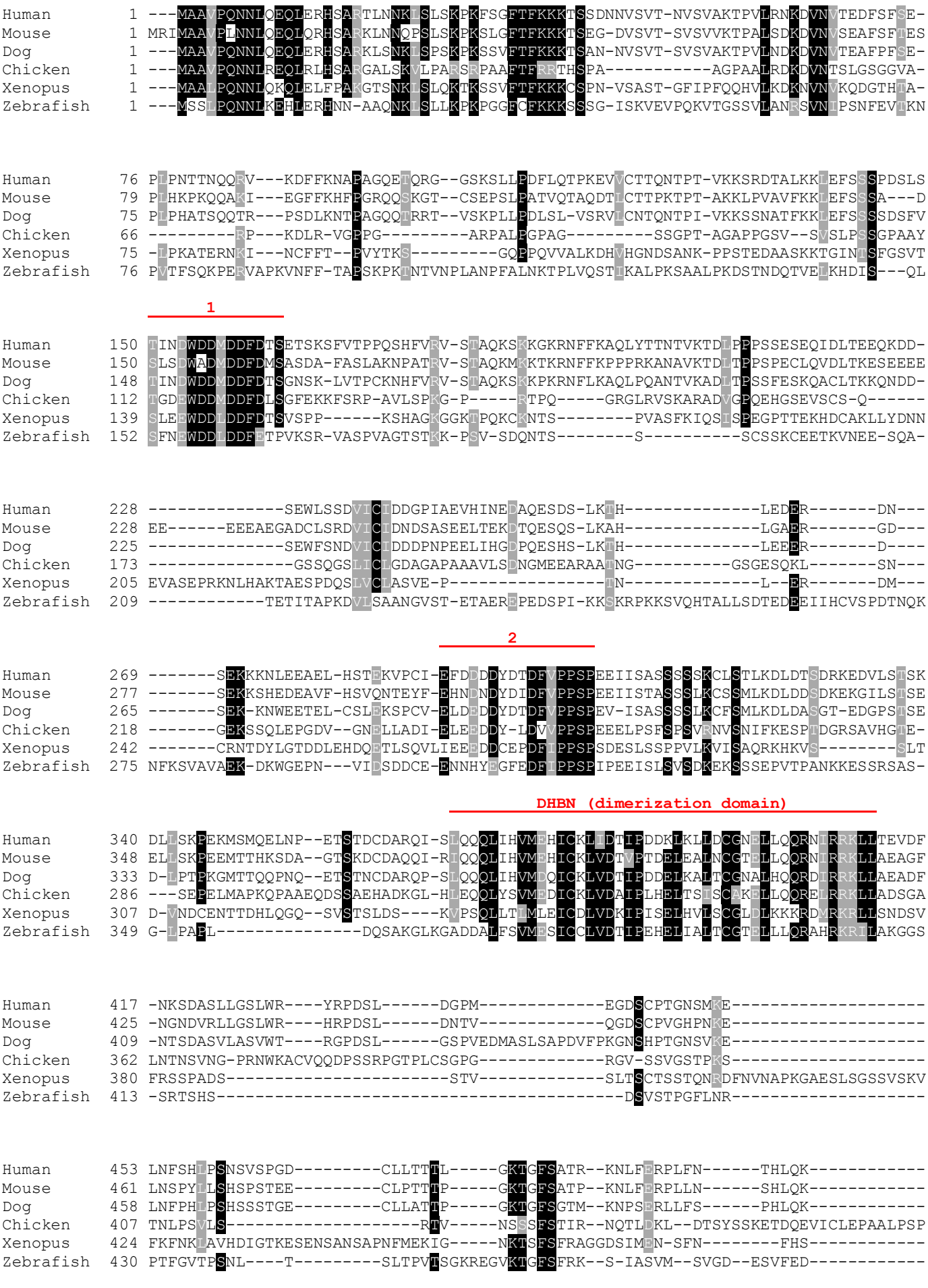
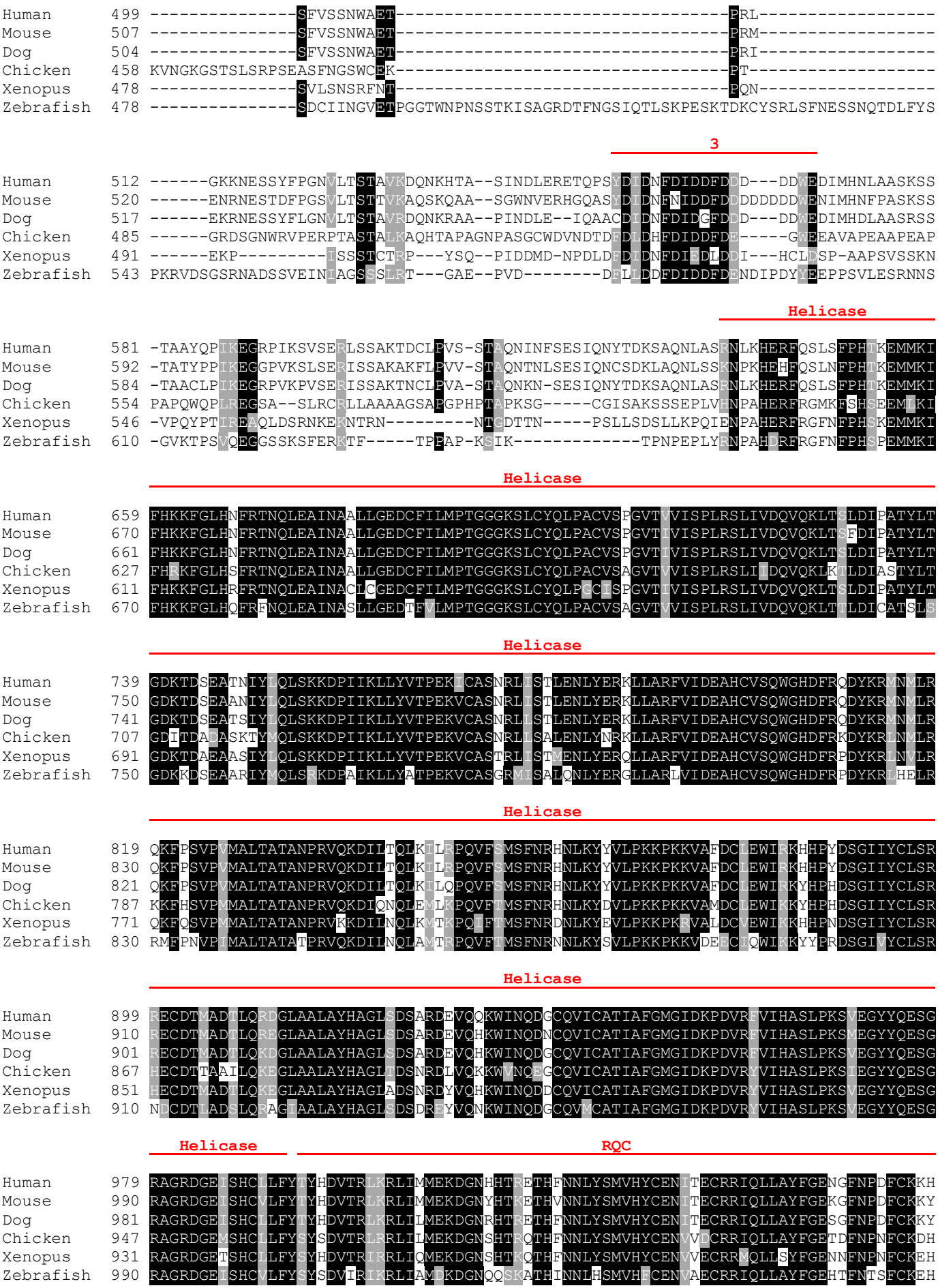


Fig. S1a

BLM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Dog 504 ----------SFVSSNWAET----------PRI----------
Chicken 458 KVNGKGSTSLSRPSEASFNGSWCEK----------PT----------
Xenopus 478 ----------SVLSNSRFNT----------PQN----------
Zebrafish 478 ----------SDCIINGVET PGGTWNPNSSTKISAGRDTFNGSIQTLSKPESKTDKCYSRLSFNESSNQTDLFYS
3
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Mouse 520 ------ENRNESTDFPGSVLTSTTVKAQSKQAA--SGWNVERHGQASYDIDNFNIDDFDDDDDDDWENIMHNFPASKSS
Dog 517 ------EKRNESSYFLGNVLTSTAVRDQNKRAA--PINDLE--IQAACDIDNFDIDGFDDD---DDWEDIMHDLAASRSS
Chicken 485 ------GRDSGNWRVPERPTASTALKAQHTAPAGNPASGCWDVNDTDFDLDHFDIDDFDE-----GWEEAVAPEAAPEAP
Xenopus 491 ------EKP---------ISSSTCTRP---YSQ--PIDDMD-NPDLDFDIDNFDIFDLDDI---HCLDSP-AAPSVSSKN
Zebrafish 543 PKRVDSGSRNADSSVEINIAGSSSLRT---GAE--PVD--------DFLLDDFDIDDFDENDIPDYYEEPPSVLESRNNS
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Dog 584 -TAACLPIKEGRPVKPVSERISSAKTNCLPVA-STAQNKN-SESIQNYTDKSAQNLASRNLKHERFQSLSFPHTKEMMKI
Chicken 554 PAPQWQPLREGSA--SLRCRLLAAAAGSAPGPHPTAPKSG-----CGISAKSSSEPLVHNPAHERFRGMKFSHSEEMIKI
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Zebrafish 610 -GVKTPSVQEGGSSKSFERKTF-----TPPAP-KSIK----------TPNPEPLYRNPAHDRFRGFNFPHSPEMMKI
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Xenopus 691 GDKTDAEAASIYIQLSKKDPIIKLLYVTPEKVCASTRLISTMENLYERQLLARFVIDEAHCVSQWGHDFRPDYKRLNVLR
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Helicase
RQC
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Chicken 947 RAGRDGEMSHCLLFYSYSDVTRLRRLILMEKDGNSHTRQTHFNNLYSMVHYCENVVDCRRIQLLAYFGETDFNPNFCKDH
Xenopus 931 RAGRDGETSHCLLFYSYHDVTRIRRLIQMEKDGNSHTKQTHFNNLYSMVHYCENVVECRRMQLLSYFGENNFNPNFCKEH
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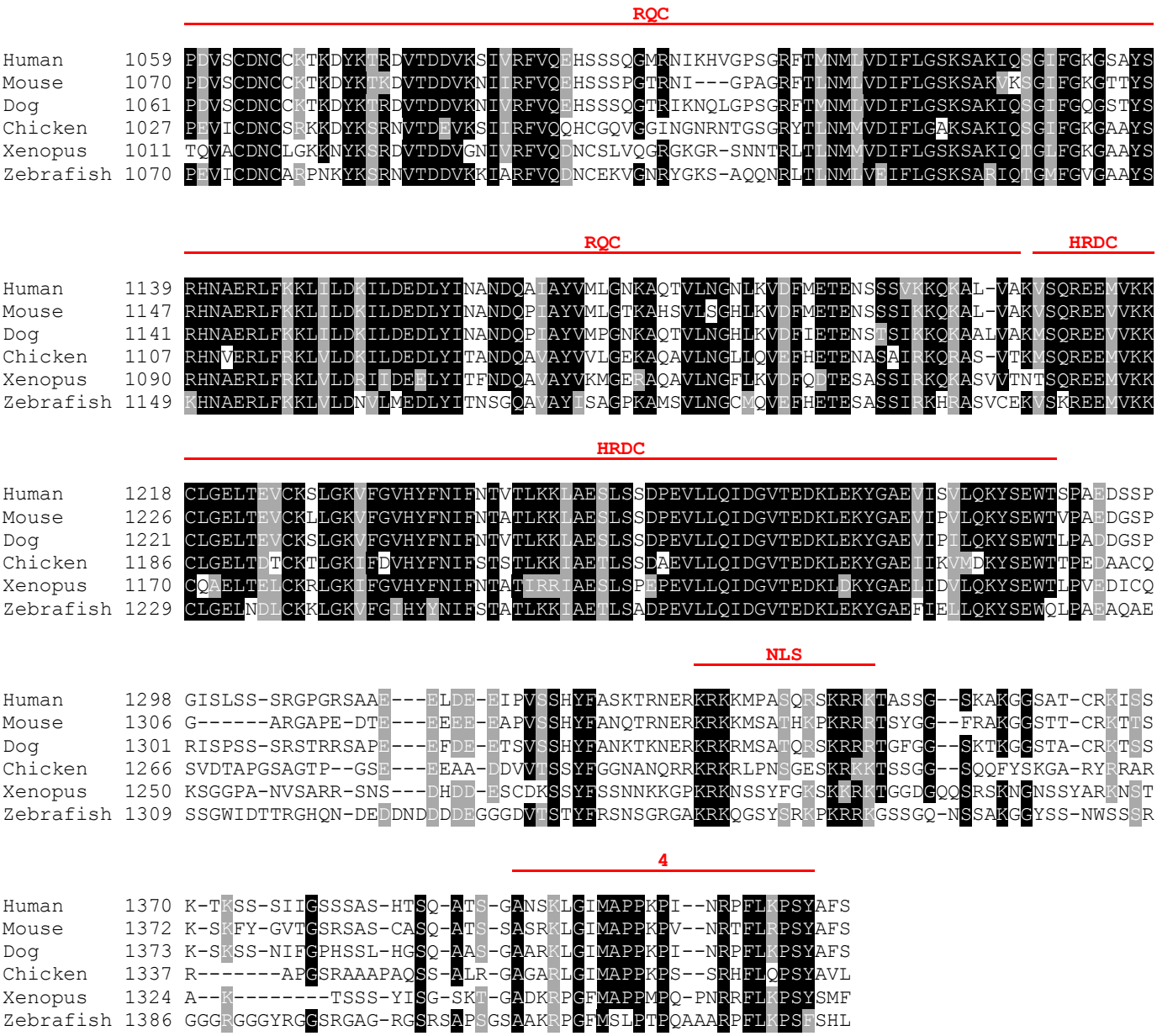
Fig. S1a

BLM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Chicken 1027 PEVICDNCSRKKDYKSRNVTDEVKSIIRFVQQHCGQVGGINGNRNTGSGRYTLNMMVDIFLGAKSAKIQSGIFGKGAAYS
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Zebrafish 1070 PEVICDNCARPNKYKSRNVTDDVKKIARFVQDNCEKVGNRYGKS-AQQNRLTLNMLVEIFLGSKSARIQTGMFGVGAAYS
RQC
HRDC
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Mouse 1147 RHNAERLFKKLILDKILDEDLYINANDQPLAYVMLGTKAHSVLSGHLKVDFMETENSSSIKKQKAL-VAKVSQREEVVKK
Dog 1141 RHNAERLFKKLILDKILDEDLYINANDQPLAYVMPGNKAQTVLNGHLKVDFLETENSTSIKKQKAALVAKMSQREEVVKK
Chicken 1107 RHNVERLFRKLVLDKILDEDLYITANDQAVAYVVLGEKAQAVLNGLLQVDFHETENASAIRKQRAS-VTKMSQREEMVKK
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Mouse 1226 CLGELTEVCKLLGKVFGVHYFNIFNTATLKKLAESLSSDPEVLLQIDGVTEDKLEKYGAEVIPVLQKYSEWTVPAEDGSP
Dog 1221 CLGELTEVCKSLGKVFGVHYFNIFNTVTLKKLAESLSSDPEVLLQIDGVTEDKLEKYGAEVIPILQKYSEWTLPADDGSP
Chicken 1186 CLGELTDTCKTLGKIFDVHYFNIFSTSTLKKIAETLSSDAEVLLQIDGVTEDKLEKYGAEIIKVMDKYSEWTTPEDAACQ
Xenopus 1170 CQAELTELCKRLGKIFGVHYFNIFNTATIRRIAESLSPEPEVLLQIDGVTEDKLDKYGAELIDVLQKYSEWTLPVEDICQ
Zebrafish 1229 CLGELNDLCKKLGKVFGIHYYNIFSTATLKKIAETLSADPEVLLQIDGVTEDKLEKYGAEFIELLQKYSEWQLPAEAQAE
NLS
Human 1298 GISLSS-SRGPGRSAAE---ELDE-EIPVSSHYFASKTRNERKRKKMPASQRSKRRKTASSG--SKAKGGSAT-CRKISS
Mouse 1306 G------ARGAPE-DTE---EEEE-EAPVSSHYFANQTRNERKRKKMSATHKPKRRRTSYGG--FRAKGGSTT-CRKTTS
Dog 1301 RISPSS-SRSTRRSAPE---EFDE-ETSVSSHYFANKTKNERKRKRMSATQRSKRRRTGFGG--SKTKGGSTA-CRKTSS
Chicken 1266 SVDTAPGSAGTP--GSE---EEAA-DDVVTSSYFGGNANQRRKRKRLPNSGESKRKKTSSGG--SQQFYSKGA-RYRRAR
Xenopus 1250 KSGGPA-NVSARR-SNS---DHDD-ESCDKSSYFSSNNKKGPKRKNSSYFGKSKKRKTGGDGQQSRSKNGNSSYARKNST
Zebrafish 1309 SSGWIDTTRGHQN-DEDDNDDDEGGGDVTSTYFRSNSGRGAKRKQGSYSRKPKRRKGSSGQ-NSSAKGGYSS-NWSSSR
4
Human 1370 K-TKSS-SIIGSSSAS-HTSQ-ATS-GANSKLGIMAPPKPI--NRPFLKPSYAFS
Mouse 1372 K-SKFY-GVTGSRSAS-CASQ-ATS-SASRKLGIMAPPKPV--NRTFLRPSYAFS
Dog 1373 K-SKSS-NIFGPHSSL-HGSQ-AAS-GAARKLGIMAPPKPI--NRPFLKPSYAFS
Chicken 1337 R-------APGSRAAAPAQSS-ALR-GAGARLGIMAPPKPS--SRHFLQPSYAVL
Xenopus 1324 A--K--------TSSS-YISG-SKT-GADKRPGFMAPPMPQ-PNRRFLKPSYSMF
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Fig. S1b

RMI1

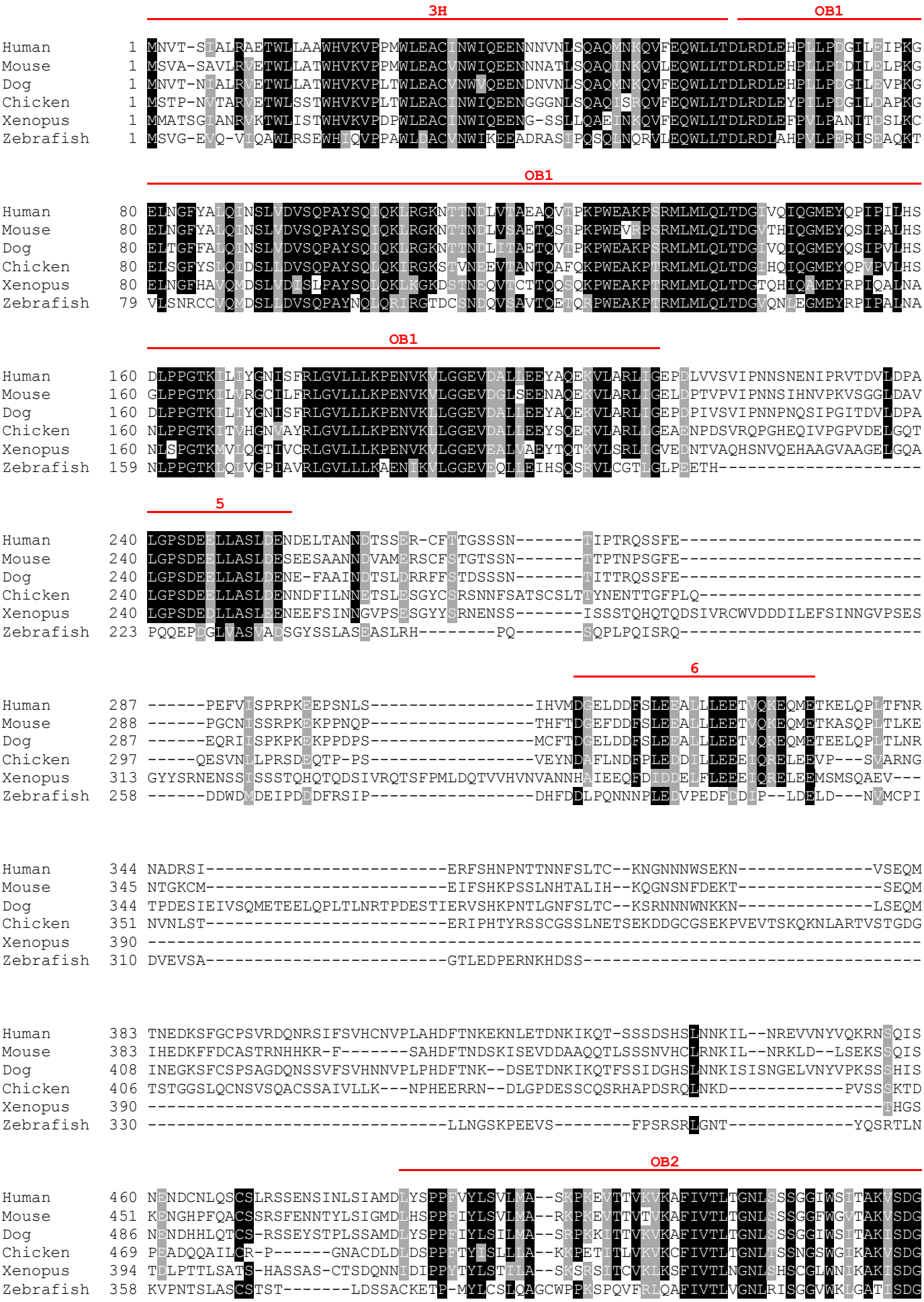
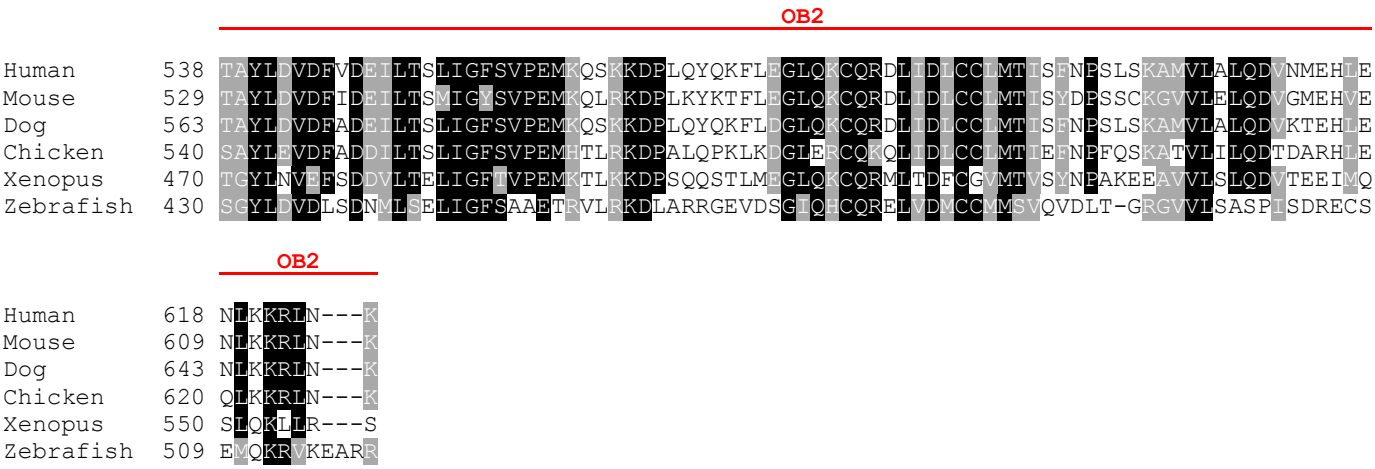

3H
OB1
OB1
OB1
5
6
OB2
Human
Mouse
Dog
Chicken
Xenopus
Zebrafish

Fig. S1b

RMI1


OB2
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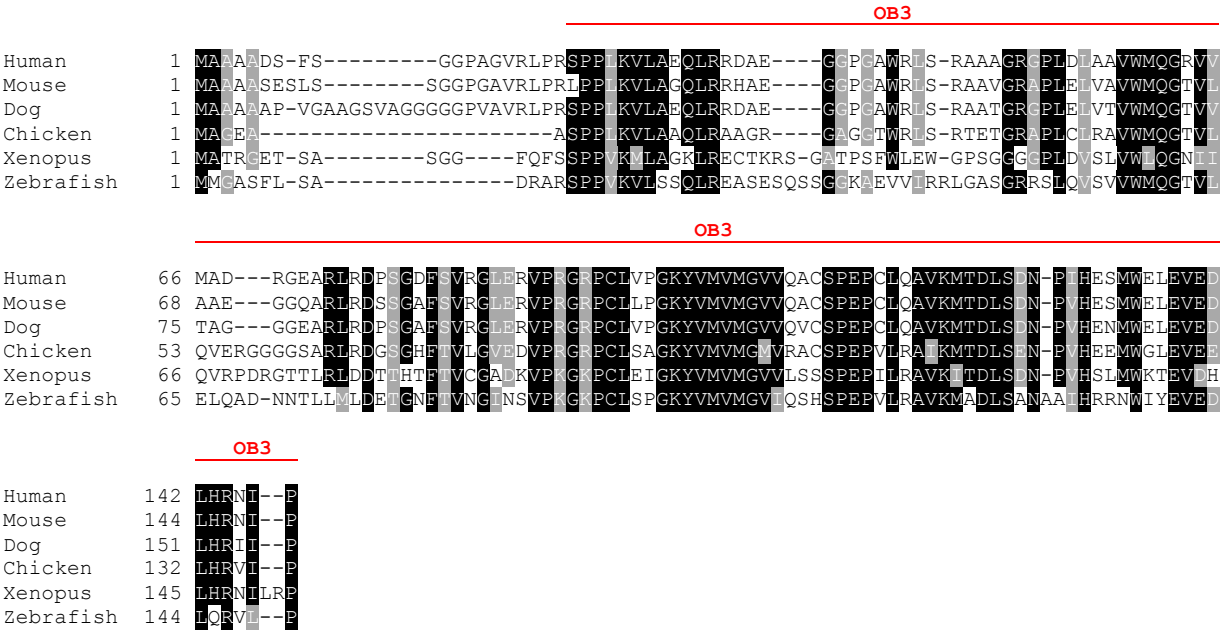
Mouse 529 TAYLDVDFIDEILTSMIGMSVPEMKQLRKDPLKYKTFLEGLQKCQRDLIDICCLMTISYDPSSCKGVVLELQDVGMEHVE
Dog 563 TAYLDVDFADEILTSLIGFSVPEMKQSKKDPLQYQKFLDGLQKCQRDLIDICCLMTISFNPSLSKAMVLALQDVKTEHLE
Chicken 540 SAYLEVDFADDILTSLIGFSVPEMHTLRKDPALQPKLKDGLERCQKQLIDICCLMTIEFNPFQSKATVLILQDTDARHLE
Xenopus 470 TGYLNVEFSDDVLTELIGFTVPEMKTLKKDPSQQSTLMEGLQKCQRMLTDFCGVMTVSYNPAKEEAVVLSLQDVTEEIMQ
Zebrafish 430 SGYLDVDLSDNMLSELIGFSAAETRVLRKDLARRGEVDSGIQHCQRELVDMCCMMSVQVDLT-GRGVVLSASPISDRECS
OB2
Human 618 NLKKRLN---K
Mouse 609 NLKKRLN---K
Dog 643 NLKKRLN---K
Chicken 620 QLKKRLN---K
Xenopus 550 SLQKLLR---S
Zebrafish 509 EMQKRVKEARR

Fig. S1c

RMI2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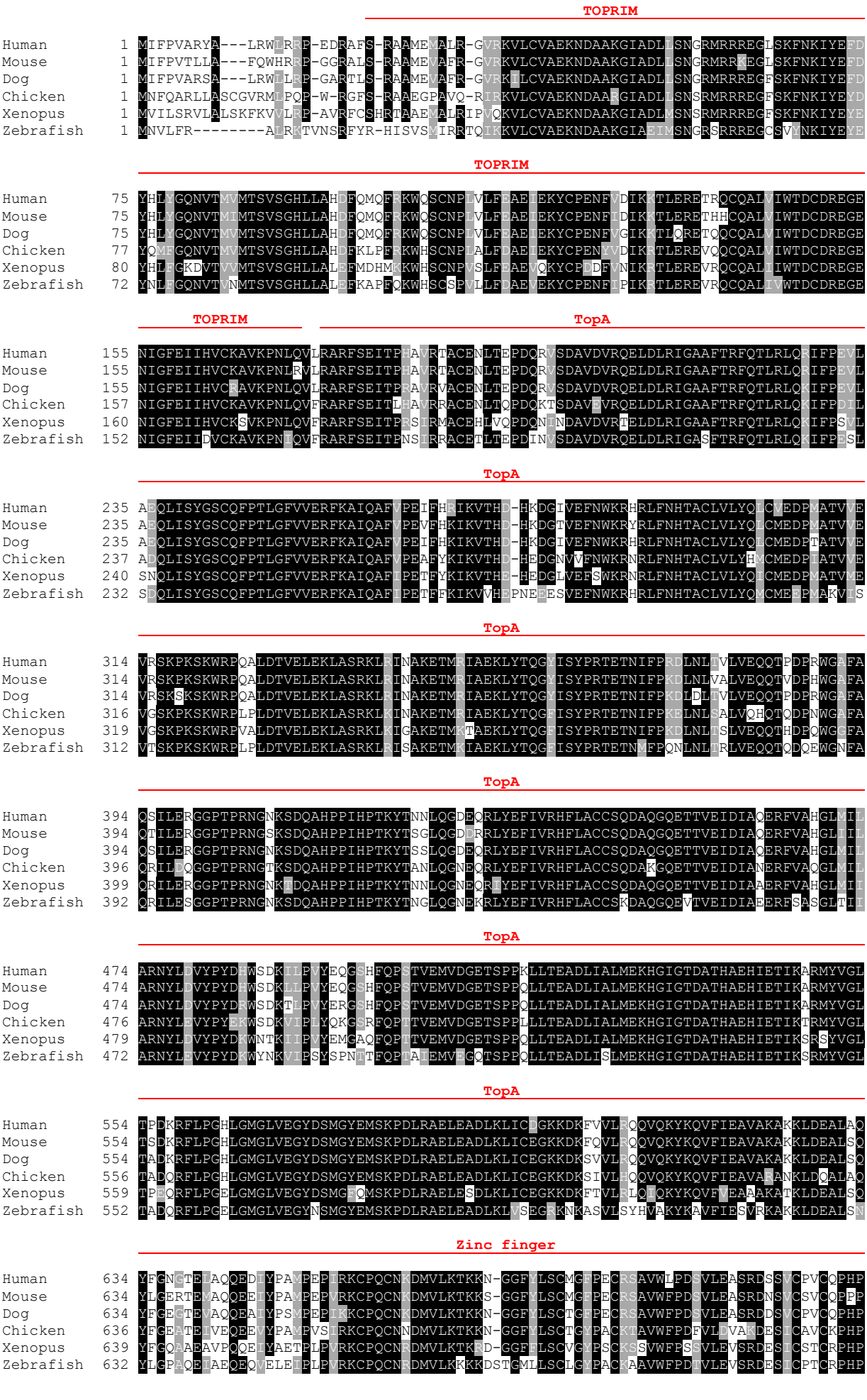
Dog 1 MAAAAAP-VGAAGSVAGGGGGPVAVRLPRSPPLKVLAEQLRRDAE----GGPCAWRLS-RAATGRGPLELVTVWMQGTVV
Chicken 1 MAGEA----------ASPPLKVLAAQLRAAGR----GAGCTWRLS-RTETGRAPLCLRAVWMQGTVL
Xenopus 1 MATRGET-SA--------SGG----FQFSSPPVKMLAGKLRECTKRS-GATPSFWLEW-GPSGGGGPLDVSLVWLQGNII
Zebrafish 1 MMGASFL-SA---------------DRARSPPVKVLSSQLREASESQSSGGKAEVVIRRLGASGRRSLQVSVVWMQGTVL
OB3
Human 66 MAD---RGEARLRDPSGDFSVRGLERVPRGRPCLVPGKYVMVMGVVQACSPEPCLQAVKMTDLSDN-PVHESMWELEVED
Mouse 68 AAE---GGQARLRDSSGAFSVRGLERVPRGRPCLLPGKYVMVMGVVQACSPEPCLQAVKMTDLSDN-PVHESMWELEVED
Dog 75 TAG---GGEARLRDPSGAFSVRGLERVPRGRPCLVPGKYVMVMGVVQVCSPEPCLQAVKMTDLSDN-PVHENMWELEVED
Chicken 53 QVERGGGGSARLRDGSGHFTVLGVEDVPRGRPCLSAGKYVMVMGMVRACSPEPVLRAIKMTDLSEN-PVHEEMWGLEVEE
Xenopus 66 QVRPDRGTTIRLDDTTHTFTVCGADKVPKGKPCIEIGKYVMVMGVVLSSSPEPILRAVKITDLSDN-PVHSLMWKTEVDH
Zebrafish 65 ELQAD-NNTLLMLDEIGNFTVNGINSVPKGKPCLSPGKYVMVMGVIQSHSPEPVLRAVKMADLSANAATHRRNWIYEVED
OB3
Human 142 LHRNI--P
Mouse 144 LHRNI--P
Dog 151 LHRII--P
Chicken 132 LHRVI--P
Xenopus 145 LHRNILRP
Zebrafish 144 LQRVL--P

Fig. S1d

TOP3A



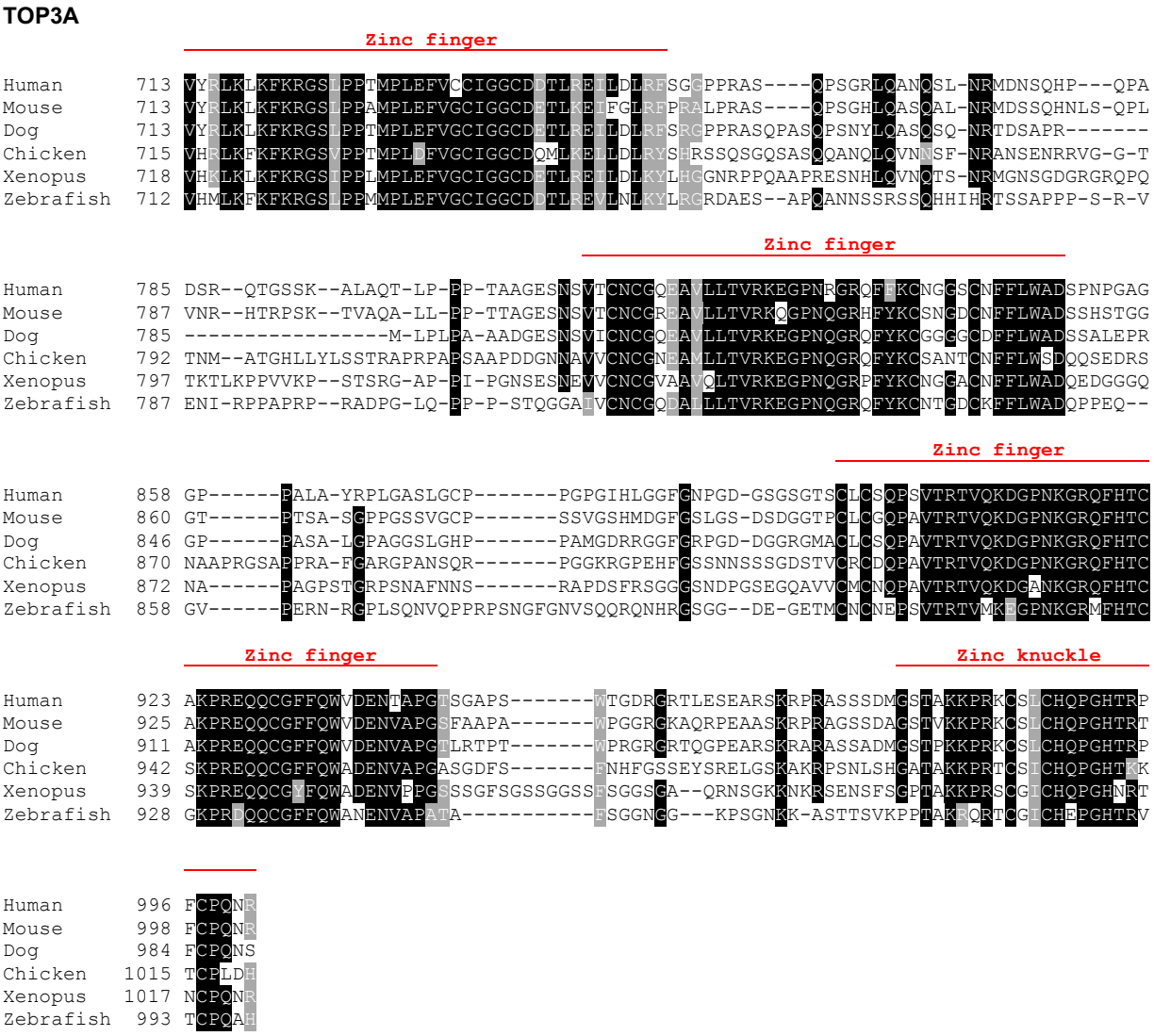


Fig. S1. Sequence alignments of (a) BLM, (b) RMI1, (c) RMI2 and (d) TOP3A orthologs from representative vertebrates. Underlined regions in red denote conserved protein domains/motifs. Numbered motifs in BLM and RMI1 correspond to sequences synthesized as biotinylated peptides (see main text for further details).

a

```
Human       1 MAAVPQNNLQEQLERHSARTLNNKLSLSKPKFSGFTFKKKTSSDNNVSVT 50
Mouse       1 MAAVPLNNLQEQLQRHSARKLNNQPSLSKPKSLGFTFKKKTSE-GDVSVT 49
Dog         1 MAAVPQNNLQEQLERHSARKLSNKLSPSKPKSSVFTFKKKTSA-NNVSVT 49
Chicken     1 MAAVPQNNLREQLRLHSARGALSKVLPARSRPAAFTFRRTHSP-AAGPAA 49
Xenopus     1 MAALPQNNLQKQLELFPAKGTSNKLSLQKTKSSVFTFKKKCSP-NVSAST 49
Zebrafish   1 MSSLPQNNLKEHLERHNN-AAQNKLSLLKPKPGGFCFKKKSSS-GISKVE 48
Sgs1        1 MVTKPSHNLRREHKWL-------KETATLQEDKDFVFQAIQKHIANKRPK 43
Δ4-37       1 MAA-----------------------------------KKKTSSDNNVSVT 16
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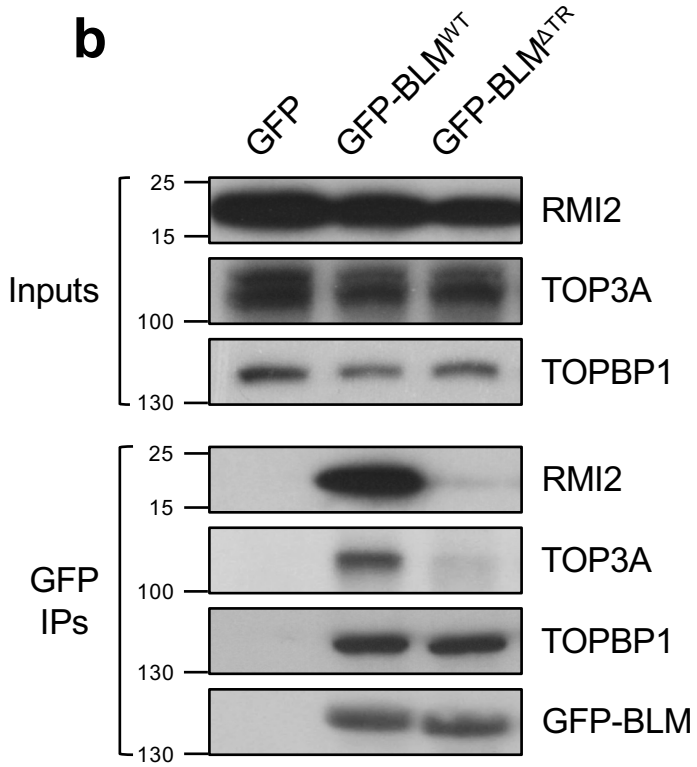

Fig. S2. Identification of a critical region required for BLM to associate with other BTR complex components.

a) Sequence alignment of the N-terminal TR region of vertebrate BLM orthologs and Sgs1. Residues in red indicate those required for Sgs1 to promote resistance to HU[31].

b) Deletion of residues 4-37 in the TR domain of BLM abrogates interaction with the BTR complex. 293FT cells were transfected with the indicated constructs and GFP-pulldowns were carried out from cell lysates, followed by western blotting for the indicated proteins.

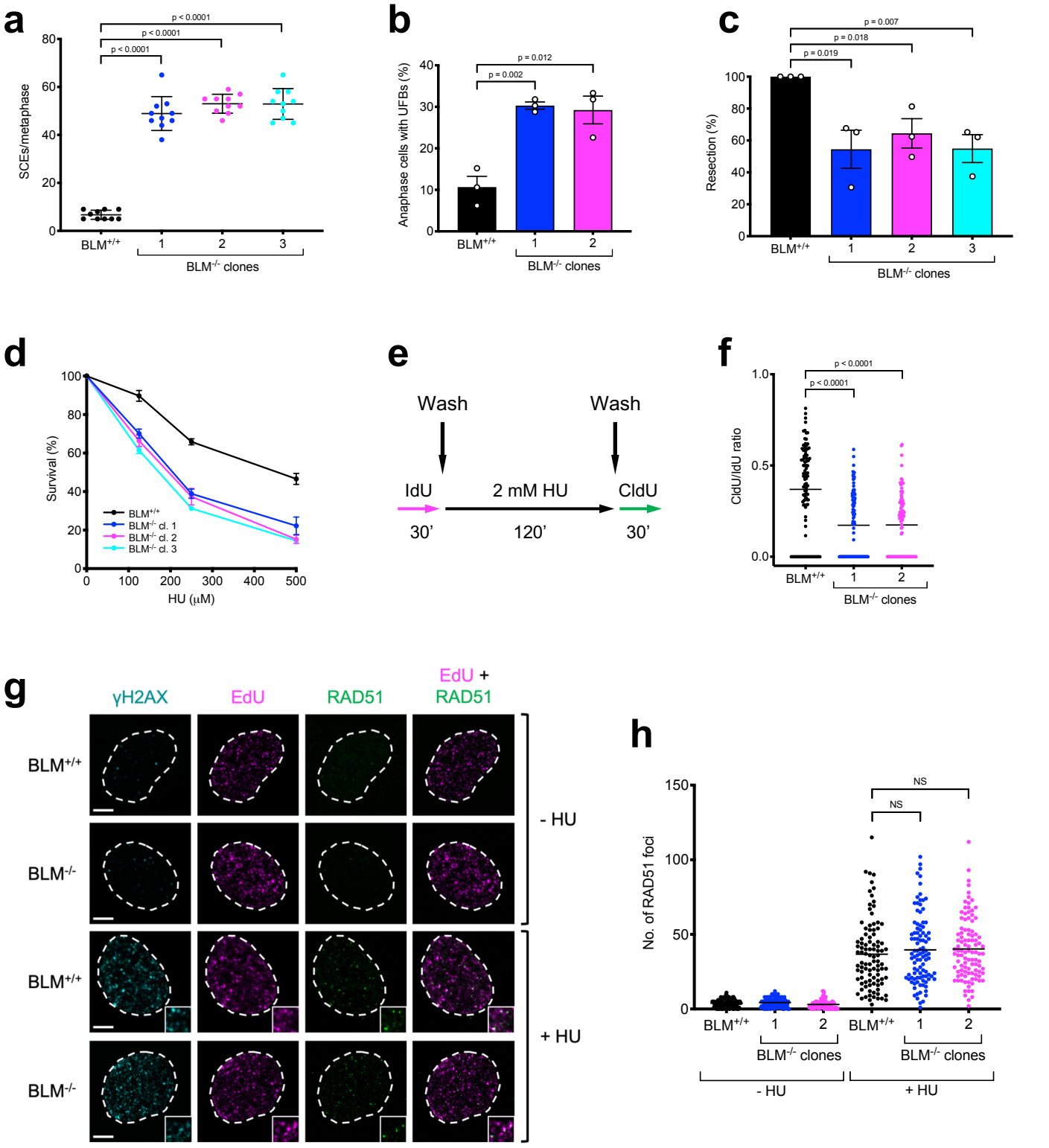
a
SCEs/metaphase
p < 0.0001
p < 0.0001
p < 0.0001
BLM+/+
1
2
3
BLM-/- clones
b
Anaphase cells with UFBs (%)
p = 0.012
p = 0.002
BLM+/+
1
2
BLM-/- clones
c
Resection (%)
p = 0.007
p = 0.018
p = 0.019
BLM+/+
1
2
3
BLM-/- clones
d
Survival (%)
BLM+/+
BLM-/- cl. 1
BLM-/- cl. 2
BLM-/- cl. 3
HU (μM)
e
Wash
Wash
IdU
2 mM HU
CldU
30'
120'
30'
f
CldU/IdU ratio
p < 0.0001
p < 0.0001
BLM+/+
1
2
BLM-/- clones
g
γH2AX
EdU
RAD51
EdU + RAD51
BLM+/+
BLM-/-
BLM+/+
BLM-/-
- HU
+ HU
h
No. of RAD51 foci
NS
NS
BLM+/+
1
2
BLM+/+
1
2
BLM-/- clones
BLM-/- clones
- HU
+ HU

Fig. S3. Characterization of *BLM*$^{-/-}$ RPE-1 cells.

a) SCE assay showing that all three *BLM*$^{-/-}$ clones have very high levels of SCEs (n=3, one representative experiment shown). At least 10 metaphases were scored per sample. Error bars denote SD. Significance was determined using the two-sided Mann-Whitney *U* test.

b) Quantification of the increase in UFBs observed in *BLM*$^{-/-}$ cells by immunofluorescence (n=3). Error bars denote SEM. Significance was determined by two-sided unpaired t test.

c) DNA-end resection is defective in *BLM*$^{-/-}$ cells as measured by RPA loading on chromatin in response to 1 μM CPT treatment for 1 hr in a FACS-based assay (n=3). Error bars denote SEM. Significance was determined by two-sided unpaired t test.

d) Colony survival assay showing that *BLM*$^{-/-}$ cells are hypersensitive to HU (n=3). Cells were treated with the indicated doses of HU for 24 hr. "cl." = clone. Error bars denote SEM.

e) Experimental outline of the DNA fibre assay to measure replication fork restart.

f) Quantification of the replication fork restart defect in *BLM*$^{-/-}$ cells by DNA fibre assay (n=2, one representative experiment shown). At least 100 DNA fibres were analysed per sample. Significance was determined using the two-sided Mann-Whitney *U* test.

g) RAD51 foci are not affected by BLM loss in RPE-1 cells. Cells were pulsed with 10 μM EdU for 15 min to mark sites of active DNA replication and treated where indicated with 2 mM HU for 2 hr. Scale bars, 5 μm.

h) Quantification of RAD51 foci in Cyclin A-positive cells, treated where indicated with 2 mM HU for 24 hr (n=2, one representative experiment shown). ~100 cells were analysed per sample. Significance was determined using the two-sided Mann-Whitney *U* test.

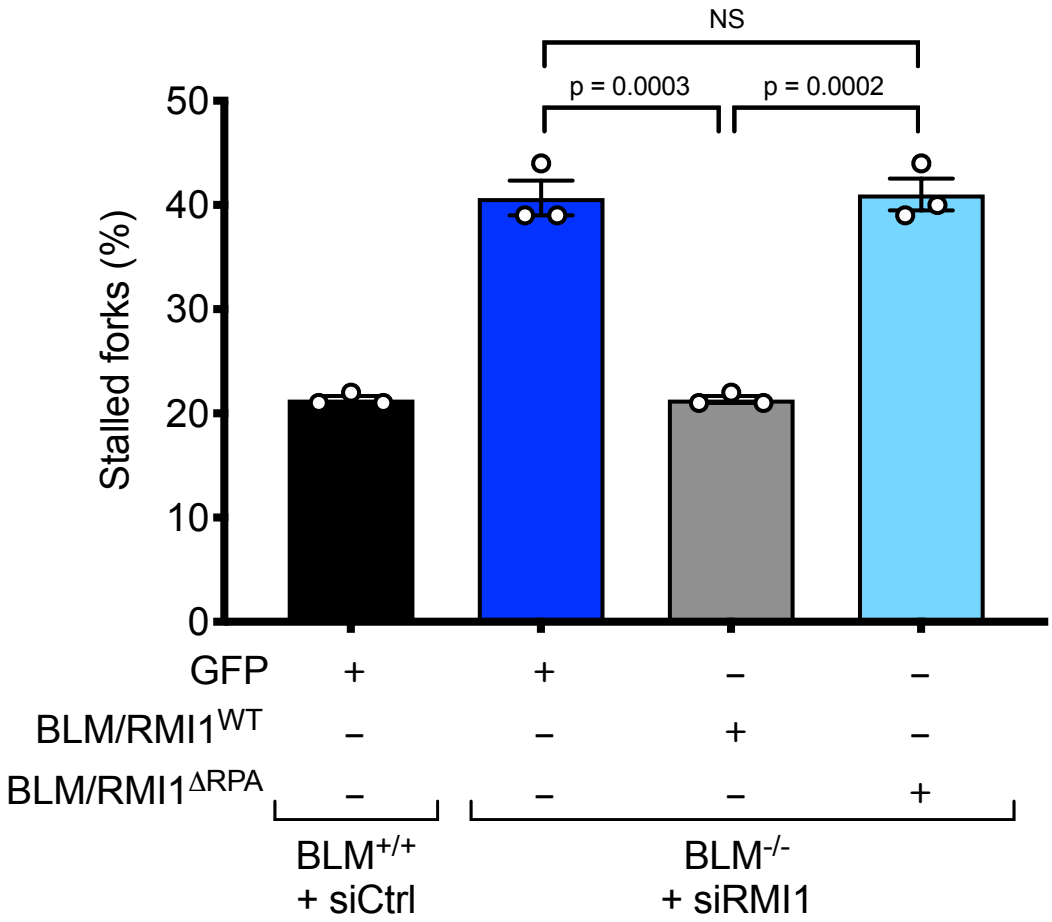

Fig. S4. RPA-binding by BLM and RMI1 suppresses increased stalled replication forks.

DNA fibre assays were carried out as in Supplementary Fig. 3e. The number of IdU-positive, CldU-negative tracts was quantified as a percentage of total fibres. At least 100 DNA fibres were analysed per sample. Significance was determined by two-sided unpaired t test (n=3, error bars denote SEM).

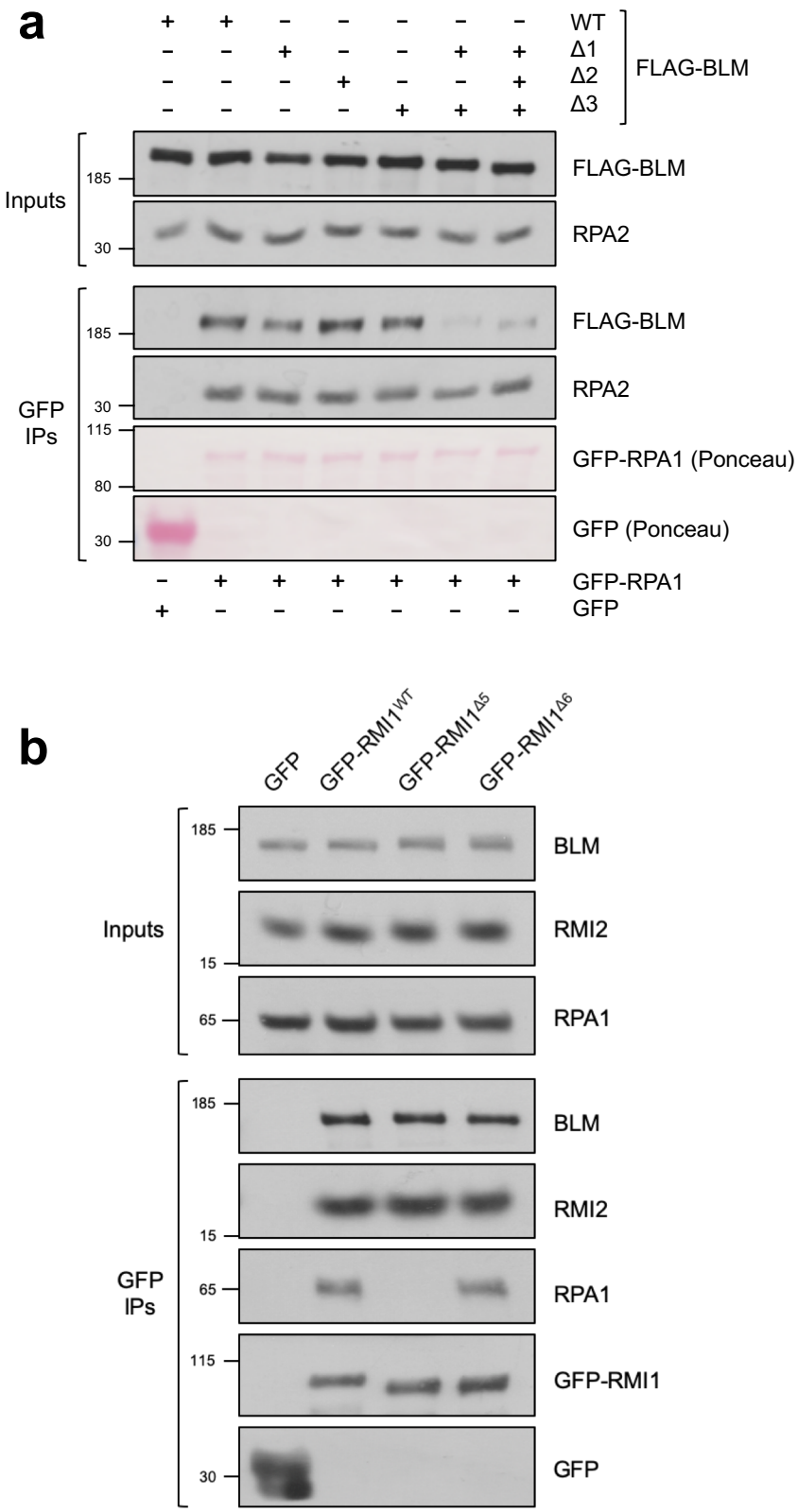

Fig. S5. BLM motif 2 and RMI1 motif 6 are not required for RPA-binding.

a) GFP-pulldowns from 293FT cells transfected with constructs expressing either GFP or GFP-RPA1, and the indicated FLAG-tagged BLM variants, showing how loss of motifs 1 and 3 but not motif 2 of BLM impacts on RPA-binding.

b) GFP-pulldowns from 293FT cells transfected with constructs expressing GFP or the indicated GFP-tagged RMI1 variants, showing that loss of motif 5 disrupts RPA-binding but motif 6 does not.

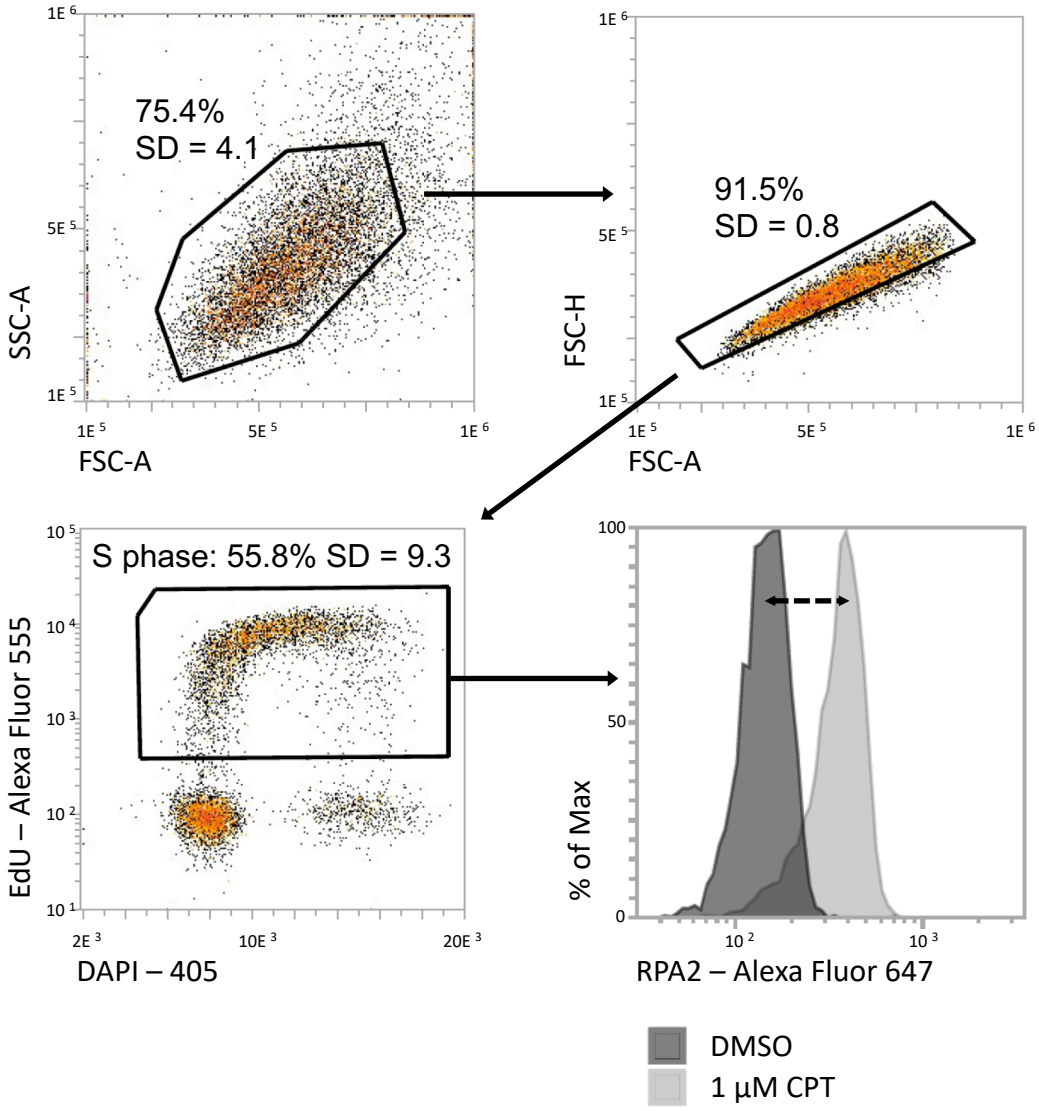

Fig. S6. Flow cytometry gating strategy.

Single RPE-1 cells were identified using Forward Scatter (FSC) and Side Scatter (SSC) and gated as indicated (top left panel, top right panel). EdU pulse-labelling was used to identify replicating cells in S phase and DAPI was used to quantify DNA content, before gating as indicated. Fluorescent marker/excitation wavelengths used are indicated on the axes (bottom left panel). Mean RPA2 intensity was calculated from the S phase population and the difference between DMSO- and CPT-treated cells found to quantify CPT-induced resection, as indicated by the dotted arrow. Fluorescent marker and excitation wavelength used are indicated on the x-axis (bottom right panel). The percentage of cells gated at each step is indicated on the panels (n = 3, SD = standard deviation).