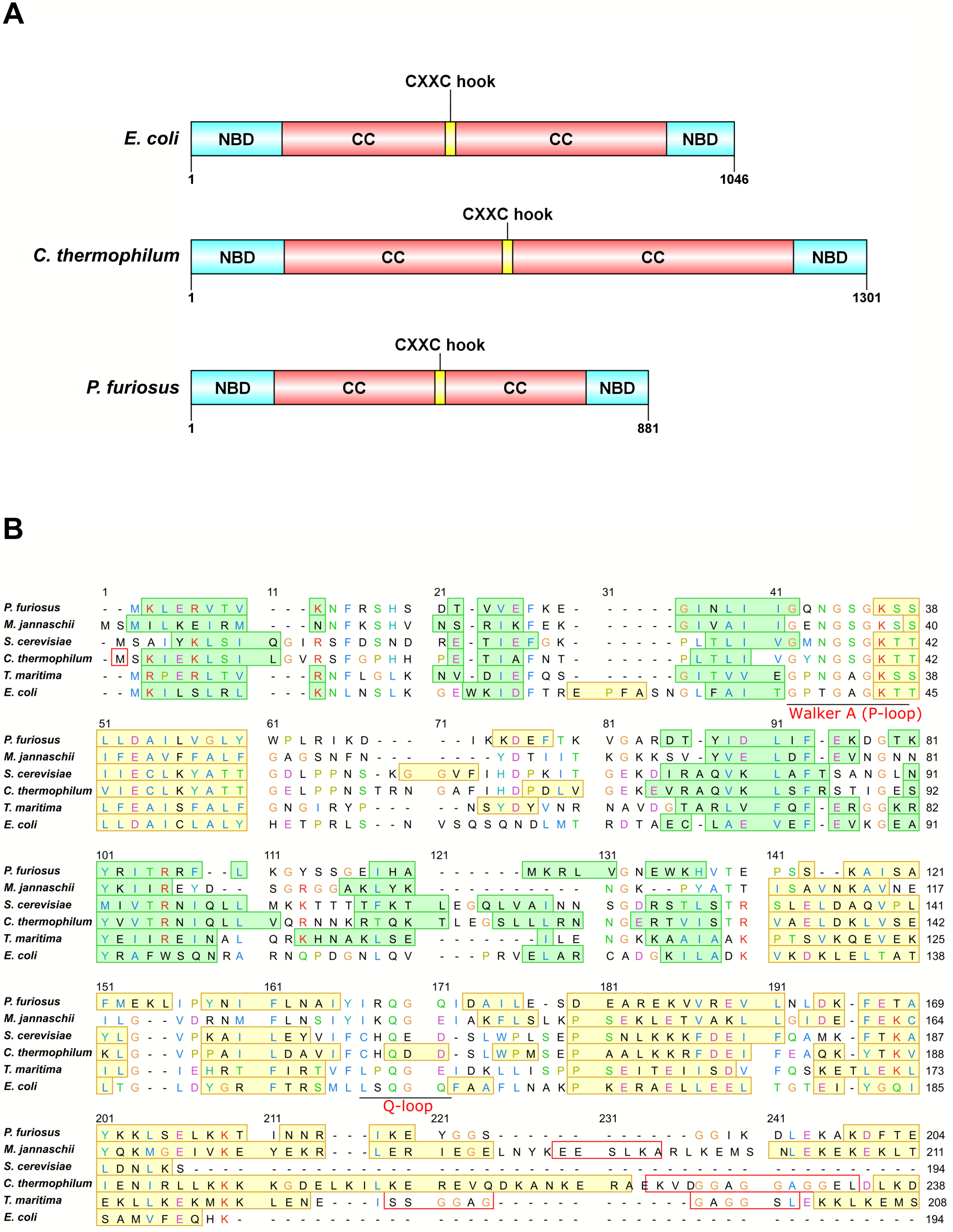
Supplementary Information

Table S1. Mutations cited in the text.

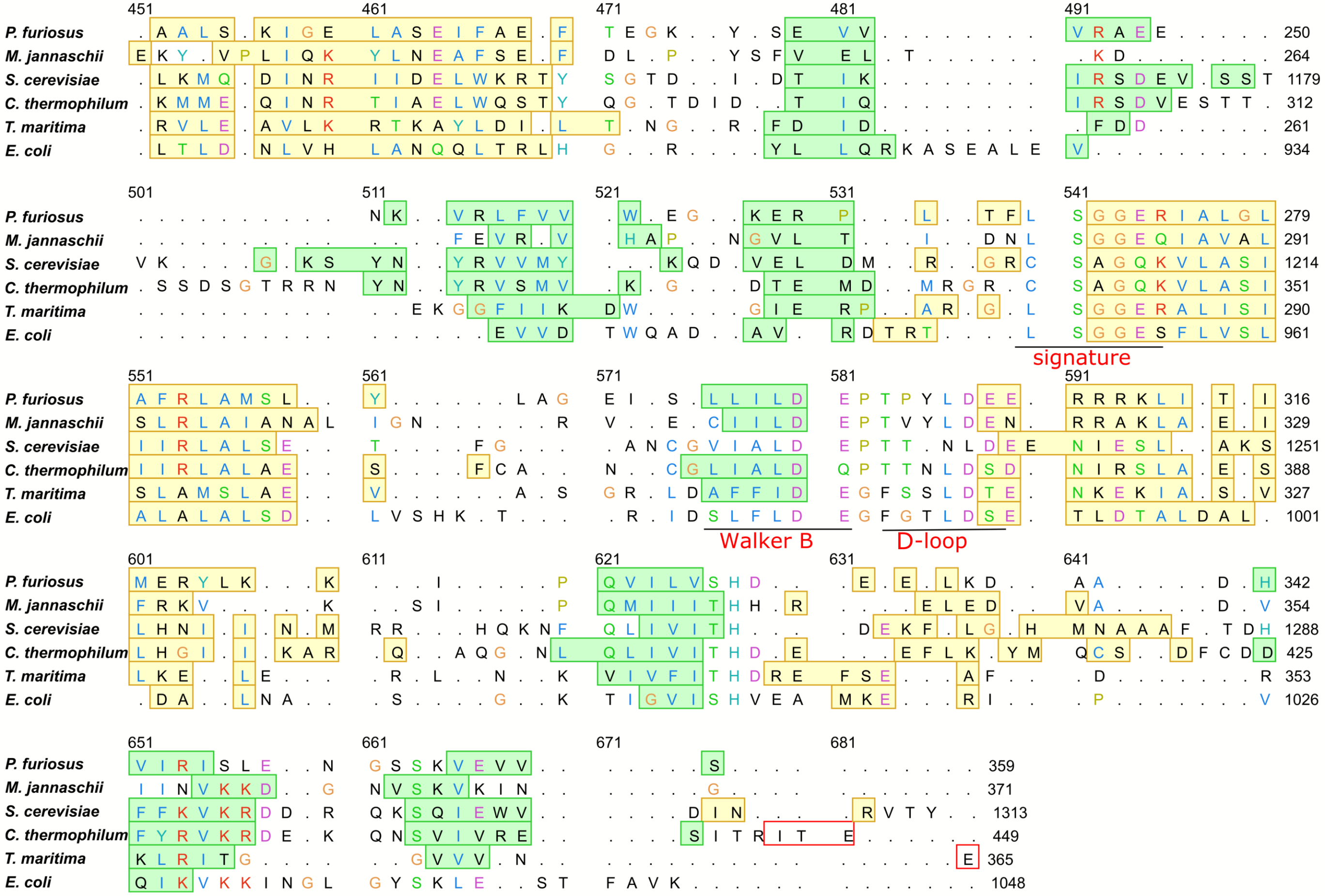
Allele	Mutation	Protein	Organism	Phenotype	Ref.
rad50-48	S679R, P682R	Rad50	*H. sapiens*	defect in Rad50 dimerization	[1]
	R805E	Rad50	*P. furiosus*	slow ATP hydrolysis	[2]
	L828F	Rad50	*P. furiosus*	defect in ATM activation	[3]
	D829N	Rad50	*P. furiosus*	defect in ATM activation	[3]
	A78T	Rad50	*S. cerevisiae*	defect in Tel1 activation	[4]
	E115K	Rad50	*E. coli*	reduced endo/exonuclease and altered DNA binding activity	[5]
rad50S	K6E	Rad50	*S. cerevisiae*	defective in removing Spo11 from DSB end	[6]
rad50S	R20M	Rad50	*S. cerevisiae*	defective in removing Spo11 from DSB end	[6]
rad50S	K81I	Rad50	*S. cerevisiae*	defective in removing Spo11 from DSB end	[6]
	Y328A	Mre11	*S. cerevisiae*	DNA damage sensitivity	[7]
	N113S	Mre11	*S. pombe*	defective Mre11 interaction with Nbs1	[8]
	S499P	Mre11	*S. cerevisiae*	defective Mre11-Rad50 interaction	[4]
	K149E	Mre11	*E. coli*	reduced endo/exonuclease and altered DNA binding activity	[5]
mre11S	T188I	Mre11	*S. cerevisiae*	defective Spo11 removal from DSB end	[9]
	R184A	Mre11	*S. cerevisiae*	DNA damage sensitive in *sgs1*Δ background	[10]
	H125N	Mre11	*S. cerevisiae*	nuclease deficient	[11]
	H52S	Mre11	*P. furiosus*	loss of exonuclease activity	[12]
	Y187C	Mre11	*P. furiosus*	loss of exonuclease activity	[13]
	R10T	Mre11	*S. cerevisiae*	*sae2*Δ DNA damage and resection defect rescue	[14]

Supplementary Figure S1

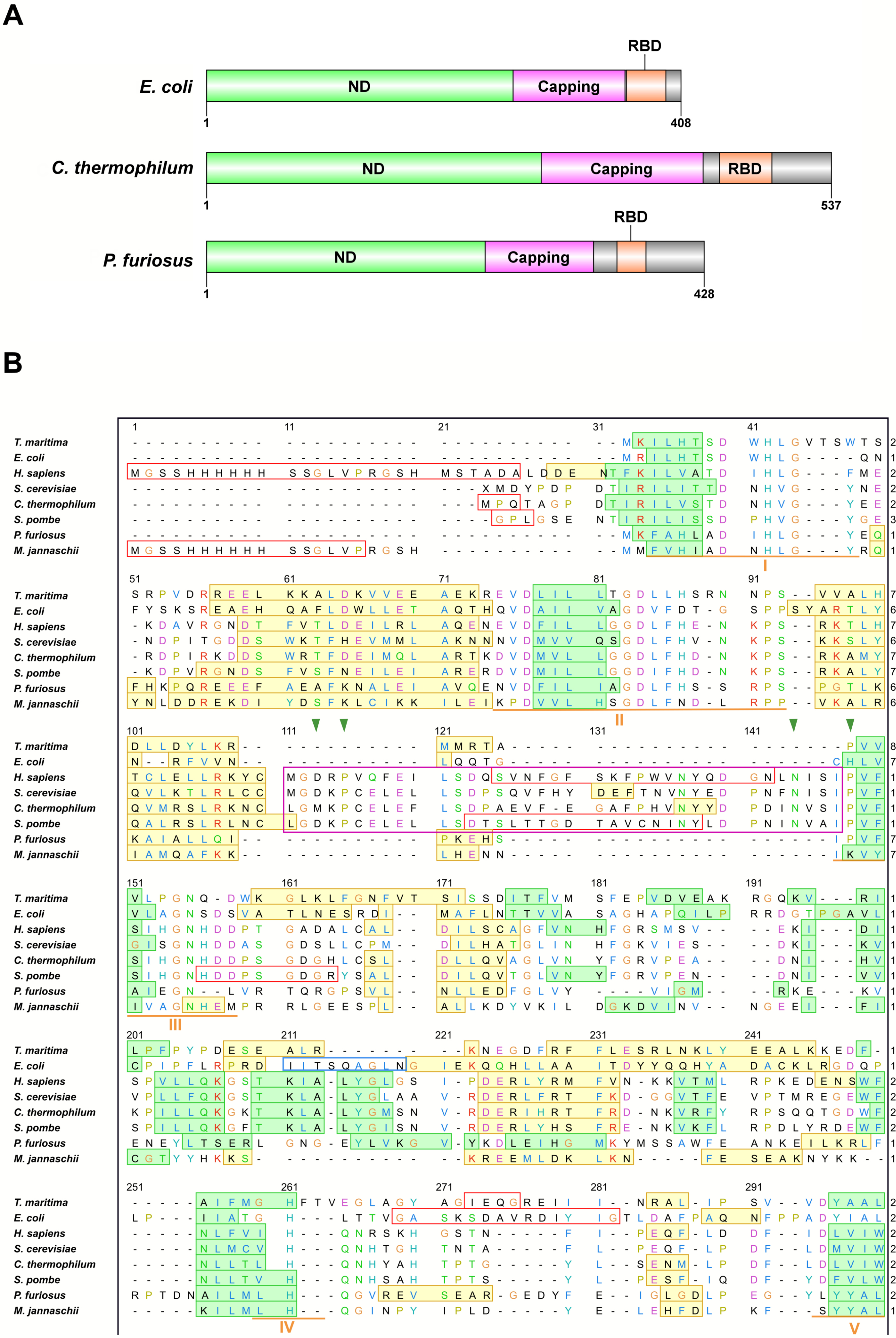


Supplementary Figure S1

C



Supplementary Figure S2



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                 1          11         21         31         41
T. maritima      ---------- ---------- ---------- --MKILHTSD WHLGVTSWTS  24
E. coli          ---------- ---------- ---------- --MRILHTSD WHLG----QN  14
H. sapiens       MGSSHHHHHH SSGLVPRGSH MSTADALDDE NTFKILVATD IHLG---FME  27
S. cerevisiae    ---------- ---------- ---XMDYPDP DTIRILITTD NHVG---YNE  23
C. thermophilum  ---------- ---------- ---MPQTAGP DTIRILVSTD NHVG---YEE  24
S. pombe         ---------- ---------- ----GPLGSE NTIRILISSD PHVG---YGE  32
P. furiosus      ---------- ---------- ---------- --MKFAHLAD IHLG---YEQ  15
M. jannaschii    MGSSHHHHHH SSGLVPRGSH ---------- --MMFVHIAD NHLG---YRQ  15

                 51         61         71         81         91
T. maritima      SRPVDRREEL KKALDKVVEE AEKREVDLIL LTGDLLHSRN NPS--VVALH  72
E. coli          FYSKSREAEH QAFLDWLLET AQTHQVDAII VAGDVFDT-G SPPSYARTLY  63
H. sapiens       -KDAVRGNDT FVTLDEILRL AQENEVDFIL LGGDLFHE-N KPS--RKTLH  73
S. cerevisiae    -NDPITGDDS WKTFHEVMML AKNNNVDMVV QSGDLFHV-N KPS--KKSLY  69
C. thermophilum  -RDPIRKDDS WRTFDEIMQL ARTKDVDMVL LGGDLFHD-N KPS--RKAMY  70
S. pombe         -KDPVRGNDS FVSFNEILEI ARERDVDMIL LGGDIFHD-N KPS--RKALY  78
P. furiosus      FHKPQREEEF AEAFKNALEI AVQENVDFIL IAGDLFHS-S RPS--PGTLK  62
M. jannaschii    YNLDDREKDI YDSFKLCIKK ILEIKPDVVL HSGDLFND-L RPP--VKALR  62

                 101        111        121        131        141
T. maritima      DLLDYLKR-- ---------- MMRTA----- ---------- -------PVV  88
E. coli          N--RFVVN-- ---------- LQQTG----- ---------- ------CHLV  78
H. sapiens       TCLELLRKYC MGDRPVQFEI LSDQSVNFGF SKFPWVNYQD GNLNISIPVF 123
S. cerevisiae    QVLKTLRLCC MGDKPCELEL LSDPSQVFHY DEFTNVNYED PNFNISIPVF 119
C. thermophilum  QVMRSLRKNC LGMKPCELEF LSDPAEVF-E GAFPHVNYYD PDINVSIPVF 119
S. pombe         QALRSLRLNC LGDKPCELEL LSDTSLTTGD TAVCNINYLD PNINVAIPVF 128
P. furiosus      KAIALLQI-- ---------- PKEHS----- ---------- ------IPVF  79
M. jannaschii    IAMQAFKK-- ---------- LHENN----- ---------- ------IKVY  79

                 151        161        171        181        191
T. maritima      VLPGNQ-DWK GLKLFGNFVT SISSDITFVM SFEPVDVEAK RGQKV---RI 134
E. coli          VLAGNSDSVA TLNESRDI-- MAFLNTTVVA SAGHAPQILP RRDGTPGAVL 126
H. sapiens       SIHGNHDDPT GADALCAL-- DILSCAGFVN HFGRSMSV-- --EKI---DI 164
S. cerevisiae    GISGNHDDAS GDSLLCPM-- DILHATGLIN HFGKVIES-- --DKI---KV 160
C. thermophilum  SIHGNHDDPS GDGHLCSL-- DLLQVAGLVN YFGRVPEA-- --DNI---HV 160
S. pombe         SIHGNHDDPS GDGRYSAL-- DILQVTGLVN YFGRVPEN-- --DNI---VV 169
P. furiosus      AIEGN--LVR TQRGPSVL-- NLLEDFGLVY ----VIGM-- --RKE---KV 114
M. jannaschii    IVAGNHEMPR RLGEESPL-- ALLKDYVKIL DGKDVINV-- NGEEI---FI 122

                 201        211        221        231        241
T. maritima      LPFPYPDESE ALR------- --KNEGDFRF FLESRLNKLY EEALKKEDF- 174
E. coli          CPIPFLRPRD IITSQAGLNG IEKQQHLLAA ITDYYQQHYA DACKLRGDQP 176
H. sapiens       SPVLLQKGST KIA-LYGLGS I-PDERLYRM FVN-KKVTML RPKEDENSWF 211
S. cerevisiae    VPLLFQKGST KLA-LYGLAA V-RDERLFRT FKD-GGVTFE VPTMREGEWF 207
C. thermophilum  KPILLQKGKT KLA-LYGMSN V-RDERIHRT FRD-NKVRFY RPSQQTGDWF 207
S. pombe         SPILLQKGFT KLA-LYGISN V-RDERLYHS FRE-NKVKFL RPDLYRDEWF 216
P. furiosus      ENEYLTSERL GNG-EYLVKG V-YKDLEIHG MKYMSSAWFE ANKEILKRLF 162
M. jannaschii    CGTYYHKKS- ---------- --KREEMLDK LKN-----FE SEAKNYKK-- 152

                 251        261        271        281        291
T. maritima      -----AIFMG HFTVEGLAGY AGIEQGREII I--NRAL-IP SV--VDYAAL 214
E. coli          LP---IIATG H---LTTVGA SKSDAVRDIY IGTLDAFPAQ NFPPADYIAL 220
H. sapiens       -----NLFVI H---QNRSKH GSTN-----F I--PEQF-LD DF--IDLVIW 243
S. cerevisiae    -----NLMCV H---QNHTGH TNTA-----F L--PEQF-LP DF--LDMVIW 239
C. thermophilum  -----NLLTL H---QNHYAH TPTG-----Y L--SENM-LP DF--LDLVIW 239
S. pombe         -----NLLTV H---QNHSAH TPTS-----Y L--PESF-IQ DF--YDFVLW 248
P. furiosus      RPTDNAILML H---QGVREV SEAR-GEDYF E--IGLGDLP EG--YLYYAL 204
M. jannaschii    -----KILML H---QGINPY IPLD-----Y E--LEHFDLP KF---SYYAL 184
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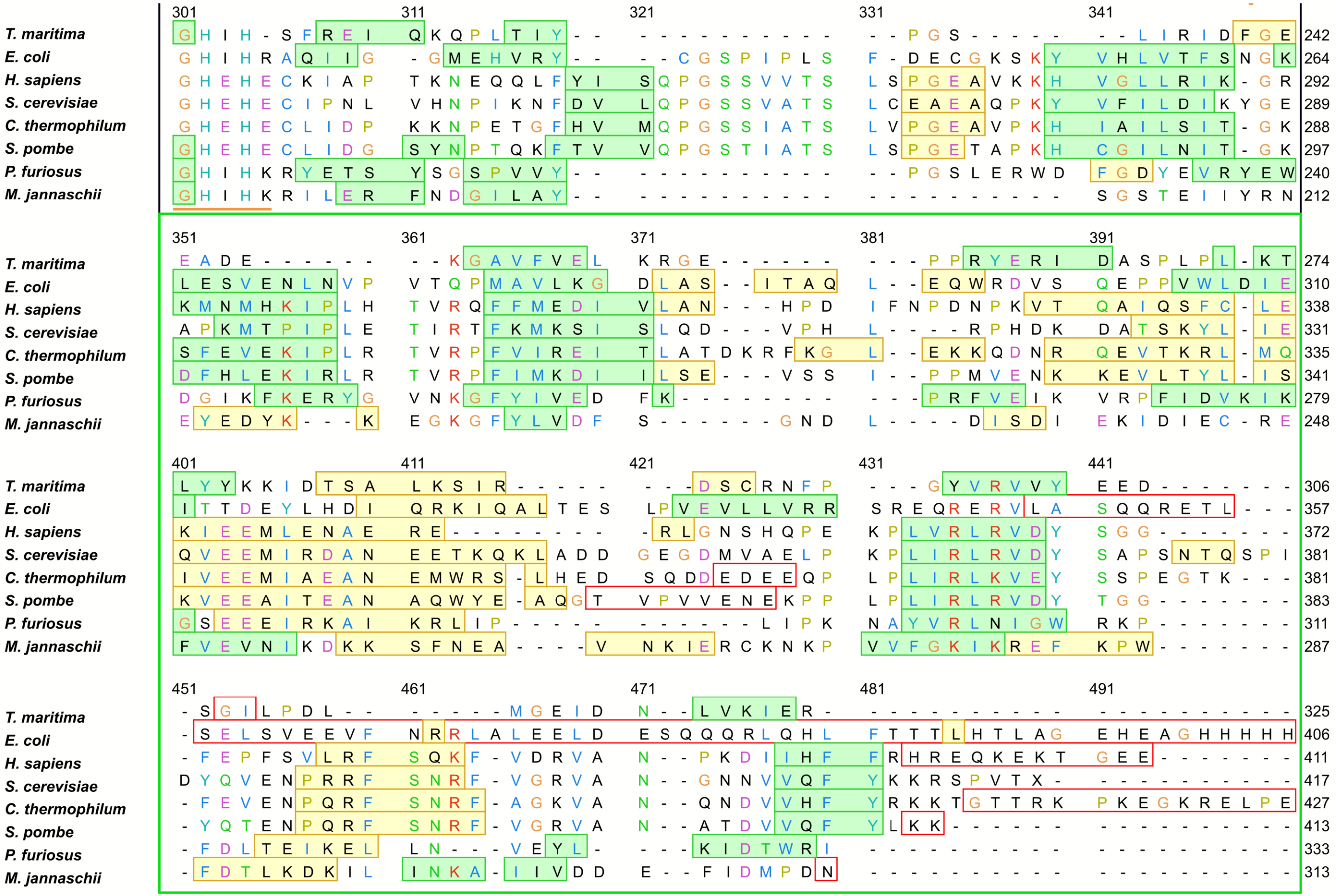
B - continued



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                 301          311          321          331          341
T. maritima      GHIH-SFREI   QKQPLTIY--   ----------   --PGS-----   --LIRIDFGE   242
E. coli          GHIHRAQIIG   -GMEHVRY--   --CGSPIPLS   F-DECGKSKY   VHLVTFSNGK   264
H. sapiens       GHEHECKIAP   TKNEQQLFYI   SQPGSSVVTS   LSPGEAVKKH   VGLLRIK-GR   292
S. cerevisiae    GHEHECIPNL   VHNPIKNFDV   LQPGSSVATS   LCEAEAQPKY   VFILDIKYGE   289
C. thermophilum  GHEHECLIDP   KKNPETGFHV   MQPGSSIATS   LVPGEAVPKH   IAILSIT-GK   288
S. pombe         GHEHECLIDG   SYNPTQKFTV   VQPGSTIATS   LSPGETAPKH   CGILNIT-GK   297
P. furiosus      GHIHKRYETS   YSGSPVVY--   ----------   --PGSLERWD   FGDYEVRYEW   240
M. jannaschii    GHIHKRILER   FNDGILAY--   ----------   ----------   SGSTEIIYRN   212

                 351          361          371          381          391
T. maritima      EADE------   --KGAVFVEL   KRGE------   ---PPRYERI   DASPLPL-KT   274
E. coli          LESVENLNVP   VTQPMAVLKG   DLAS--ITAQ   L--EQWRDVS   QEPPVWLDIE   310
H. sapiens       KMNMHKIPLH   TVRQFFMEDI   VLAN---HPD   IFNPDNPKVT   QAIQSFC-LE   338
S. cerevisiae    APKMTPIPLE   TIRTFKMKSI   SLQD---VPH   L----RPHDK   DATSKYL-IE   331
C. thermophilum  SFEVEKIPLR   TVRPFVIREI   TLATDKRFKG   L--EKKQDNR   QEVTKRL-MQ   335
S. pombe         DFHLEKIRLR   TVRPFIMKDI   ILSE---VSS   I--PPMVENK   KEVLTYL-IS   341
P. furiosus      DGIKFKERYG   VNKGFYIVED   FK--------   ---PRFVEIK   VRPFIDVKIK   279
M. jannaschii    EYEDYK---K   EGKGFYLVDF   S------GND   L----DISDI   EKIDIEC-RE   248

                 401          411          421          431          441
T. maritima      LYYKKIDTSA   LKSIR-----   ---DSCRNFP   ---GYVRVVY   EED-------   306
E. coli          ITTDEYLHDI   QRKIQALTES   LPVEVLLVRR   SREQRERVLA   SQQRETL---   357
H. sapiens       KIEEMLENAE   RE--------   -RLGNSHQPE   KPLVRLRVDY   SGG-------   372
S. cerevisiae    QVEEMIRDAN   EETKQKLADD   GEGDMVAELP   KPLIRLRVDY   SAPSNTQSPI   381
C. thermophilum  IVEEMIAEAN   EMWRS-LHED   SQDDEDEEQP   LPLIRLKVEY   SSPEGTK---   381
S. pombe         KVEEAITEAN   AQWYE-AQGT   VPVVENEKPP   LPLIRLRVDY   TGG-------   383
P. furiosus      GSEEEIRKAI   KRLIP-----   ------LIPK   NAYVRLNIGW   RKP-------   311
M. jannaschii    FVEVNIKDKK   SFNEA----V   NKIERCKNKP   VVFGKIKREF   KPW-------   287

                 451          461          471          481          491
T. maritima      -SGILPDL--   -----MGEID   N--LVKIER-   ----------   ----------   325
E. coli          -SELSVEEVF   NRRLALEELD   ESQQQRLQHL   FTTTLHTLAG   EHEAGHHHHH   406
H. sapiens       -FEPFSVLRF   SQKF-VDRVA   N--PKDIIHF   FRHREQKEKT   GEE-------   411
S. cerevisiae    DYQVENPRRF   SNRF-VGRVA   N--GNNVVQF   YKKRSPVTX-   ----------   417
C. thermophilum  -FEVENPQRF   SNRF-AGKVA   N--QNDVVHF   YRKKTGTTRK   PKEGKRELPE   427
S. pombe         -YQTENPQRF   SNRF-VGRVA   N--ATDVVQF   YLKK------   ----------   413
P. furiosus      -FDLTEIKEL   LN---VEYL-   ---KIDTWRI   ----------   ----------   333
M. jannaschii    -FDTLKDKIL   INKA-IIVDD   E--FIDMPDN   ----------   ----------   313
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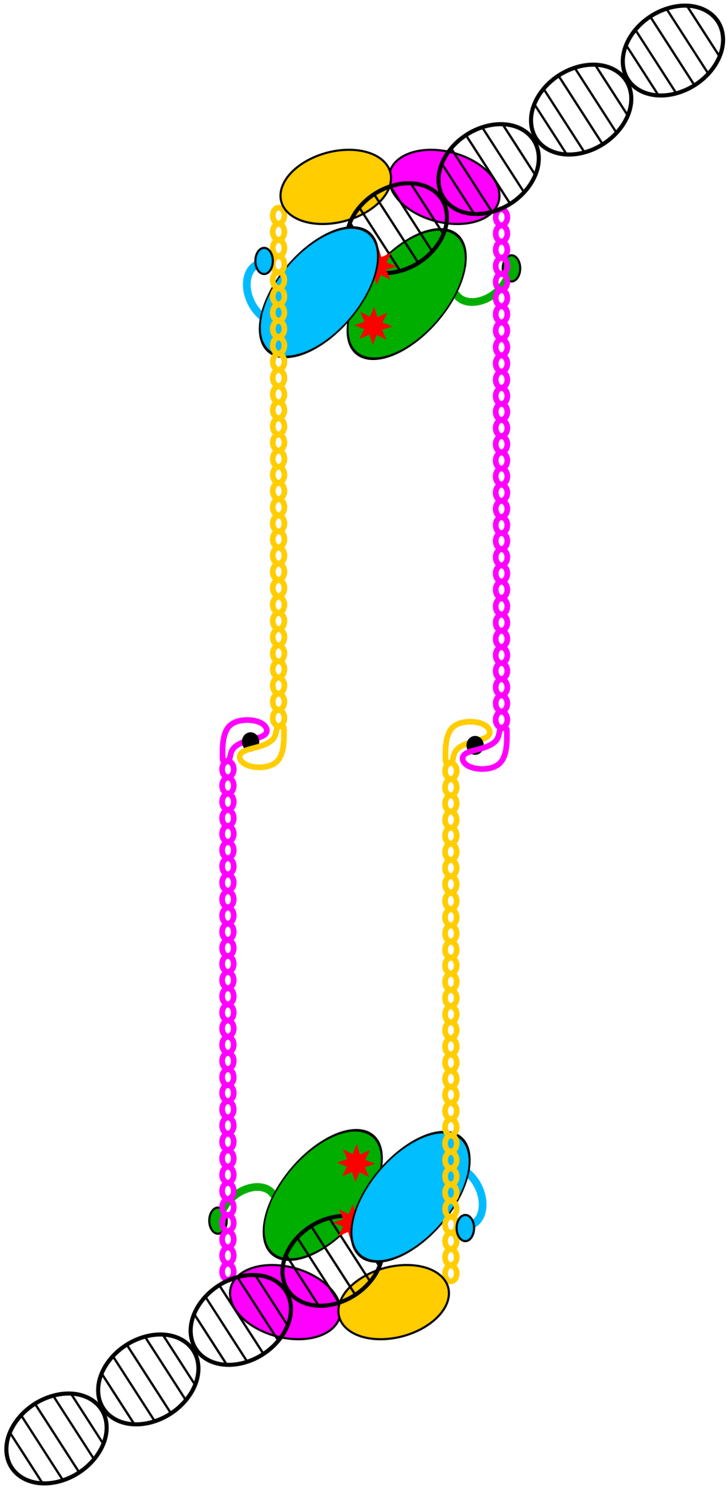
Supplementary Figure S3



Supplementary figure legends

Supplementary Figure S1. Structural features and structural alignment of Rad50 orthologs. (A) Scheme depicting the conserved domains present in Rad50 orthologues from the indicated organisms. (B,C) Structural alignment of the lobe I (B) and lobe II (C) of Rad50 orthologues from eubacteria *T. maritima* (PDB ID: 4W9M) and *E. coli* (PDB ID: 6S6V), eukaryotes *S. cerevisiae* (model from reference [4]) and *C. thermophilum* (PDB ID:5DAC), and archaea *P. furiosus* (PDB ID:3QKU) and *M. jannaschii* (PDB ID:3AV0). Secondary structures are indicated, wherever the structure is available (otherwise the sequence is marked by a red rectangle), as follows: solid yellow rectangles, alpha helix; solid green rectangles, beta sheets. Alignment residues coloring is according to ClustalW style. Conserved functionally relevant motifs are underlined and labelled.

Supplementary Figure S2. Structural features and structural alignment of Mre11 orthologs. A. Scheme depicting the conserved domains present in Mre11 orthologs from the indicated organisms. (B) Structural alignment of the N-terminal regions of Mre11 orthologues from eubacteria *T. maritima* (PDB ID: 4NZV) and *E. coli* (PDB ID: 6S6V), eukaryotes *S. cerevisiae* (model from reference [15]), *H. sapiens* (PDB ID:3T1I), *C. thermophilum* (PDB ID:4KYE), and *S. pombe* (PDB ID:4FCX), and archaea *P. furiosus* (PDB ID:1II7) and *M. jannaschii* (PDB ID:3AV0). Secondary structures are indicated, wherever the structure is available (otherwise the sequence is marked by a red rectangle), as follows: solid yellow rectangles, alpha helix; solid green rectangles, beta sheets. Alignment residues coloring is according to ClustalW style. Functional regions of the proteins are indicated by open boxes: black box, endonuclease domain; green box, capping domain; purple box, latching loop; blue box, fastener loop. Orange bars highlight conserved phosphoesterase signature motifs (I to V). Green triangles indicate the residues identified for Nbs1 binding in *S. pombe* [8].

Supplementary Figure S3. DNA tethering can be achieved by bridging the DSB ends through an intercomplex dimerization at the Zn-hook interface of two distinct MR complexes.

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