1	Co-expression and purification of the RadA recombinase with the
2	RadB paralog from <i>Haloferax volcanii</i> yields multimeric ring
3	structures.
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5	
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9	
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### 2 Supplementary Methods and Figures

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## 4 Hvo RadA-RadB complex did not form a complex with DNA in different trials

DNA binding of Hvo RadB over-expressed in E. coli is already established (GUY et al. 2006). 5 6 However under the same reaction conditions, the RadA-RadB complex did not show detectable protein-DNA complex formation with either single stranded (ELB 40 (70 mer)) or a partial 7 8 duplex (ELB 40/ELB 41 (33 mer)) previously used analysis with the Hvo RadB protein. The 9 assay was also performed with salt concentrations ranging from 0.1 M to 1 M NaCl or KCl in the binding buffer. Replacing magnesium with an EDTA-based buffer and adding ATP in the 10 11 binding buffer with increasing concentration of the RadA-RadB protein complex (0-8  $\mu$ M) did 12 not result in protein-DNA complex formation on either 10% or 7% polyacrylamide.

13 During this study, E. coli RecA (2 µM) was also used as a positive control in the same binding buffer used for Hvo RadA-B complex with the exclusion of salt (i.e. containing 20 mM Tris pH 14 7.5, 15 mM MgCl<sub>2</sub>, 2 mM DTT, 0.05 mg/ml BSA, 6% glycerol, 2 mM non-hydrolyzable ATP 15 analog). Hvo RPA3 at concentrations ranging from 3-9 µM was assayed for DNA binding in 16 17 varying NaCl/KCl (0.1-1 M) and MgCl<sub>2</sub> (15-30 mM) concentrations. Addition of salt is generally considered unfavorable for agarose gel electrophoresis as this may cause overheating 18 19 due to the high conductivity of salt-containing buffers. Limiting NaCl concentration up to 0.4 M 20 was found to be compatible when gels and buffers were pre-cooled at  $4^{0}$ C; a distinct nucleoprotein complex was observed for Hvo RPA3 in the given conditions. Electrophoretic 21 Mobility Shift Assay (EMSA) of Hvo RPA3 using  $\Phi$ X174 ssDNA in a horizontal agarose gel 22 23 (0.6%) was also optimized to a NaCl/KCl concentration ranging from 0.4 M to 1 M KCl. Protein-DNA complexes were not observed for the RadA-B complex in contrast with Hvo RPA3 24 for which protein-DNA interaction was detected under similar conditions (WINTER et al. 2012). 25

Fluorescence polarisation anisotropy was attempted to obtain quantitative analysis of the Hvo RadA-RadB complex as this assay had previously been used successfully to demonstrate Hvo RPA3 DNA binding in high salt concentrations up to 3 M KCl (WINTER et al. 2012). The assay

- 1 was performed using an 18mer oligonucleotide labelled at the 5' end with a Cy5 fluorophore in
- 2 the presence of 1-3 M KCl. No DNA binding to the Hvo RadA-B complex was detected.

DNA binding assay	DNA substrate	Protein concentration	Binding buffer components	Incubation time and temperature	Running Buffer
EMSA: Agarose gel (0.6%) retardation assay	500 ng of PhiX174 ssDNA (NEB)	0-10 μM	20mM Tris, 15mM or 30 mM MgCl2, 2mM DTT, 50 μg/mL BSA, 6% glycerol, 2 mM non- hydrolyzable ATP, and KCl concentration (0.2, 0.4, 0.5 or 1 M)	10 minutes at 37 °C or 40 °C.	1x TBE buffer
EMSA: Polyacrylamide TBE gel (10%)	0.2 nM (ELB40) 70- mer ssDNA or a partial duplex of ELB 40/ELB 41 (33 mer)	0-8 μM	20 mM Tris pH7.5, 15 mM MgCl <sub>2</sub> , 2 mM DTT, 50 µg/mL BSA, 6% glycerol, 2 mM non- hydrolyzable ATP, and 0.4 M or 1 M NaCl.	10 minutes at 37 °C or 40 °C.	1x TBE buffer
Fluorescence polarisation anisotropy	20nM 18mer Cy5- fluorophore labelled oligonucleotide at the 5' end	0-8 μM	50mM HEPES pH 7.0, 10% glycerol, 0.03% BSA, 2 mM non- hydrolyzable ATP. KCl concentration either 1, 2 or 3 M.		

Table S1. Different methods employed for DNA binding assay using purified RadA-RadB
 complex

#### 2 Supplementary Figure legends

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## 3 Figure S1. Sequence alignment of recombination proteins in different domains of life.

S1A. A cartoon representation showing the structural comparison of *P. furiosus* (Pfu) RadA
(1pzn) to eukaryotic Rad51 (1szp) and *E.coli* RecA (2reb). Pfu RadA is shown in blue,
eukaryotic Rad51 in red and RecA is colored yellow. Pfu RadA shows similarity in the ATPase
domain (AD) to both eukaryotic Rad51 and RecA. Pfu RadA possesses N-terminal domain
(ND) like eukaryotic Rad51, with bacterial RecA instead contains C-terminal domain (CTD),
colored yellow.

S1B. Amino acid sequence alignment of Hvo RadA with RadA homologues. Sequence alignment demonstrates the conservation of the ATP (Walker A and B) and DNA (L1 and L2) binding motifs, indicated in green and blue respectively. Highly conserved residues are highlighted in red. Abbreviations: *Methanococcus voltae* (Mvo), *Pyrococcus furiosus* (Pfu); *Archaeoglobus fulgidus* (Afu); *Sulfolobus solfataricus* (Sso); *Saccharomyces cerevisae* (Sc); *Homo sapiens* (Hs).

16 **S1C.** Comparison of RadA and RadB domain structure (adapted from (KOMORI *et al.* 2000)).

Green boxes represent the Walker A and Walker B motifs, respectively, in the conserved ATPase
domain (RadA-c). RadB lacks the N-terminal domain (RadA-n) and contains the conserved
ATPase domain.

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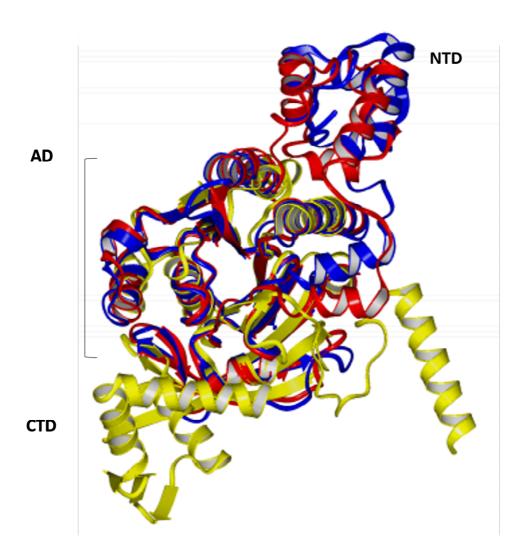
Figure S2. Selected amino acid usage in *H. volcanii* RadA compared with RecA/Rad51 homologs from other species. Sequence identification Accession numbers (NCBI) of each protein: Hvo RadA (ELY24318.1); Hs Rad51 (AFN04713.1); Mvo RadA (AAC23499.1); Afu RadA (KUK05438.1); Pfu RadA (AFN04713.1); Ec RecA (AFU91764.1). Amino acid quantitation of selected amino acids of RadA or homologues from different species was computed using ExPASy ProtParam and is presented as a relative percentage.

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Figure S3. EMSA at 0.4 M KCl shows DNA binding by Hvo RPA3 but not the RadA-B complex. The Hvo RPA3 protein has been previously shown to form protein-DNA complexes

1	under in vitro conditions (Winter et al., 2012). Therefore RPA3 was used as a positive control.
2	RPA3 and RadA-B complex samples were pre-incubated with <sup>32</sup> P-labeled DNA at 2 nM final
3	concentration in binding buffer supplemented with 0.4 M NaCl at 42 °C, for 10 min. Protein-
4	DNA complexes were analysed by 10% TBE gel in TBE buffer. Increasing protein concentration
5	results in increased complex formation for RPA3, whereas no binding was observed for the
6	RadA-B complex.
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- Figure S1
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#### Unconserved 012345678910 Conserved

	10	20	30	40	50			
Hvo_RadA								
MCh_RadA					MAGE			
Hs Bad51					MAGE			
Sc Rad51	MSQVQEQHIS	ESQLOYGNGS	LMSTVPADLS	QSVVDGNGNG	SSEDIEATNG			
Sso_RadA								
Consistency	0000000000	0000000000	0000000000	0000000000	0000000000			
	60	70						
			MAE	DDLESLPG DNLTDLPG	VGPATADKLV VGPSTAEKLV			
	EVKEIDEFEE	LGEEPATEET	PKKKKKEKII	RSIEDLPG	VGPATAEKLR			
Hs_Rad51	SGDGGGLQEQ	LEANADTSV-	<mark>EEESF</mark> GP	QP ISR LEQCG	INANDVKKLE			
Sc_Rad51	SGDGGGLQEQ	AEAQGEMEDE	AYDEAALGSF	VP IEK LQVNG	I TMADVKKLR			
Sso_RadA	MSNEVE		QKKNI	KTINDLPG	ISQTVINKLI			
Consistency	0000111113	1001001100	0001011132	3 4 <mark>8 5 4 <mark>-</mark> 6 5 0 0</mark>	<mark>9 5 4 5 5 6 5 * *</mark> 3			
		0 12	0 13	0 14	0 150			
Hyo Rada	ESGYDSYQSI		KADIGSSTAS		DVGGEETGSM			
	EAGYIDEMKI	ATATVGELTD	IEGISEKAAA	KMIMGARDLC	DLG-FKSGID			
Pfu_RadA	EAGYDTLEAI	AVASPIELKE	VAGISEGTAL	KIIQAARKAA	NLGTEMRADE			
	EAGFHTVEAV	AYAPKKELIN	IKGISEAK <mark>a</mark> d	KILAE <mark>A</mark> AKLV	PMG-FTTATE			
Sc_Rad51	ESGLHTAEAV	AYAPRKDLLE	IKGISEAKAD	<b>KLLNEAARLV</b>	PMG-FVTAAD			
	EAGYSSLETL * 8 * 6 3 6 4 6 5 8	AVASPQDLSV - 5 - 5438 - 44	AAGIPLSTAQ 557 - 6555 - 3	KIIKEARDAL 78835 • 6565	$\frac{\mathbf{D}\mathbf{IR} - \mathbf{F}\mathbf{K}\mathbf{TALE}}{5770 \cdot 46735}$			
Consistency		5438444	557 6555 3	7 8 8 3 5 6 5 6 5 Walker A	5//046/35 motif			
	16	0 17	0 18		0			
Hvo_RadA	VLERBOOIGK	LSWQIDEVDE	LLGGGLETQS	ITEVYGEEGA	GKSQITHQLA			
Mth_RadA	LLKQRSTVWK	LSTSSSELDS	VLGGGLESQS	VTE <mark>FAGVF</mark> GS	GKTQIMHQSC			
	YLKKRATIGR	ISTGSKS LDK	LL <mark>G</mark> GGIET <mark>Q</mark> A	ITEVFGEFGS	GKTQL <mark>A</mark> HTLA			
Hs_Rad51	FHQRRSEIIQ	ITTCSKELDK	LLQCCIETCS	ITEMFCEFRT	CKTQICHTLA			
Sc_Rad51	FHMRRSELIC	LTTGSKNLDT	LL <mark>G</mark> GGVET <mark>G</mark> S	ITELFGEFRT MTEFFGEFGS	GKSQL <mark>C</mark> HTLA			
Sso_RadA	VKKERMNVKK 5356 54825	ISTGSQALDG 87767558 • 4	LLAGGIETRT 8 * 5 * * 8 * 8 4 7	8 * * 5 6 * 7 * 5 6	GKT <mark>QLC</mark> HQLS = = 7 = 8 <mark>4</mark> = 67 6			
Consistency	555654625	0//0/550-4	0 - 5 - 0 - 0 • 7	<u> </u>				
		0	0	0 24	0			
Hvo_RadA	VNVQLPPEQ-	G	GLGGGCIFID	SEDTFRPERI	DDMVRGLEDE			
Mth_RadA	VNLQNPEFLF	YDEEAVSKG <mark>E</mark>	VAQPKAVY ID	TEGTFRPERI	MOMAEHA			
Pfu_RadA	VMVQLPPEE-	<mark>c</mark>	GLNGSVIWID	TE <mark>n</mark> tfrperi	REIAQNR			
	VTCOLPIDR-	G	G <mark>ge</mark> gkamy I D	TEGTFRPERL	LAVAERY			
Sc_Rad51 Sso_RadA	VTCQIPLDI- VNVQLPPEK-	G	GGEGKCLYID GLSGKAVYID	TEGTFRPVRL TEGTFRWERI	VSIAQRF Enmakal			
	55 6 3530		74476577**	8 - 6 67 - 8	3478633000			
		0 27		0	0			
	ALEATLDDRE		I KALVDDFLD	KI HVAKAFNS	NH <mark>QILLAEK</mark> A			
Mth_RadA Pfu RadA			- GIDGQTVLD - GLDPDEVLK	NTFVARAYNS HIYVARAFNS	DMOMLFAEKI			
			-GLSGSDVLD	NVAYARAFNS				
Sc Rad51			-GLDPDDALN	NVAYARAYNA	DHOLRLLDAA			
Sso_RadA			- GLDIDNVMN	NITTIRAINT	DHQIAIVDDL			
	0000000000	0000000000	0775365695	674578 6 6	77 5476546			
		Walker B mo		Loop 1				
					0			
HVO_RadA	KELAGEHEDT EDLIQEGN	NIKLVVID	SLTAHFRAEY SLTSTFRNEY	TGRGE LAERQ	QKLNKHLHDL QKLGRHMATL			
Pfu Rada	EDKIKELLNT	DRPVKLLIVD	SLTSHFRSEY	IGRGALAERO	QKLGRHMATL QKLAKHLADL			
	SAMMVES	RYALLIVD	SATALYRTDY	SGRGELSARQ	MHLARFLRML			
Sc_Rad51	AQMMSES	<mark>RFS</mark> LIVVD	S VMALYRTDF	SGRGELSARQ	MH <mark>LA</mark> KFMRAL			
Sso_RadA	QELVSKDP	<mark>sik</mark> livvd	SVTSHFRAEY	PGREILAVRO	QKLNKHLHQL			
Consistency	5566 <mark>383000</mark>	00365 879 *	677 <mark>3</mark> 8 588	4 7 4 8 5	76486843			
	36	0	0. Loop 2 38	0				
Hyo Rada			PDSYFGD	0				
Mth Rada	NKLADLENCY	VLVTNQVSAK	PDAFFGM	AEQAIGGHIV	GHAATERFFV			
Pfu_RadA	NKLADLFNCV HRLANLYDIA	VEVINOVQAR	PDAFFGD	PTRPIGGHIL	ABSATLRVYL			
	LRLADEFGVA	VVITNOVVAQ	VDGAAMFAAD	PKKPIGGNII	AHASTTRLYL			
Sc_Rad51	QRLADQF GVA	VVVTNOVVAQ	VDGGMAENPD	PKKPIGGNIM	A <mark>H</mark> SSTT <mark>RLGF YHVPGIRIQL</mark>			
Sso_RadA	TRLAEVYDIA	VI I TNOVMAR	PDMFYGD 5 - 30018457	P TVAVGGHTL	YHVPGIRIQL			
Consistency	y <mark>3 8 9 8 7 4 8</mark> 5 5 6	9 <mark>69****</mark> 485	5 3 0 0 1 8 4 5 7	7 <mark>5 5 6 9 * *</mark> 6 7 7	5 <mark>- 5 6 7 4 - 6 4</mark> 7			
Hvo Rada	RKSKGDKRIV	RLVDAPNLAD	GEAIMRVQDA					
	RKGKGDKRVA	KLYDSPHLPD	AEAIFRITEK	GIQD				
Pfu_RadA	RKGKGGKRIA	RLIDAPHLPE	GEAVFSITEK	GIQD				
	RKGR <mark>g</mark> etric	KIYDSPCLPE	AEAMPAINAD	GVGDAKD	_			
Sc_Rad51	KKGK <mark>C</mark> QRLC	KVVDSPCLPE	AECVFAIYED	GVGDPREEDE				
Sso_RadA	<b>KKSRGNRRIA</b>	RVVDAPHLPE	GEVVFALTEE	GIRDAEE				
Consistency	y 8 = 7 8 = <mark>3 5</mark> = 8 <mark>5</mark>	776 = 7 = 4 = 78	6 = 6 8 8 <mark>5 8 4 6 4</mark>	<b>8</b> 47111000				

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