SUPPLEMENTARY MATERIALS

*Correspondence should be addressed to K.K.
E-mail: komatsu@house.rbc.kyoto-u.ac.jp

The proteasome factor Bag101 binds to Rad22 and suppresses homologous recombination

Yuichiro Saito[1], Jun Takeda[2], Masahiro Okada[3], Junya Kobayashi[1], Akihiro Kato[1], Kouji Hirota[2], Masato Taoka[2], Tomohiro Matsumoto[4], Kenshi Komatsu[1*] and Toshiaki Isobe[2]

[1] Division of Genome Repair Dynamics, Radiation Biology Center, Kyoto University, Yoshida-konoecho, Sakyo-ku, Kyoto 606-8501, Japan.

[2] Department of Chemistry, Tokyo Metropolitan University, Minami-Osawa, Hachioji-shi, Tokyo 192-0397, Japan.

[3] Center for Priority Areas, Tokyo Metropolitan University, Minami-Osawa, Hachioji-shi, Tokyo 192-0397, Japan.

[4] Division of System biology, Radiation Biology Center, Kyoto University, Yoshida-konoecho, Sakyo-ku, Kyoto 606-8501, Japan.

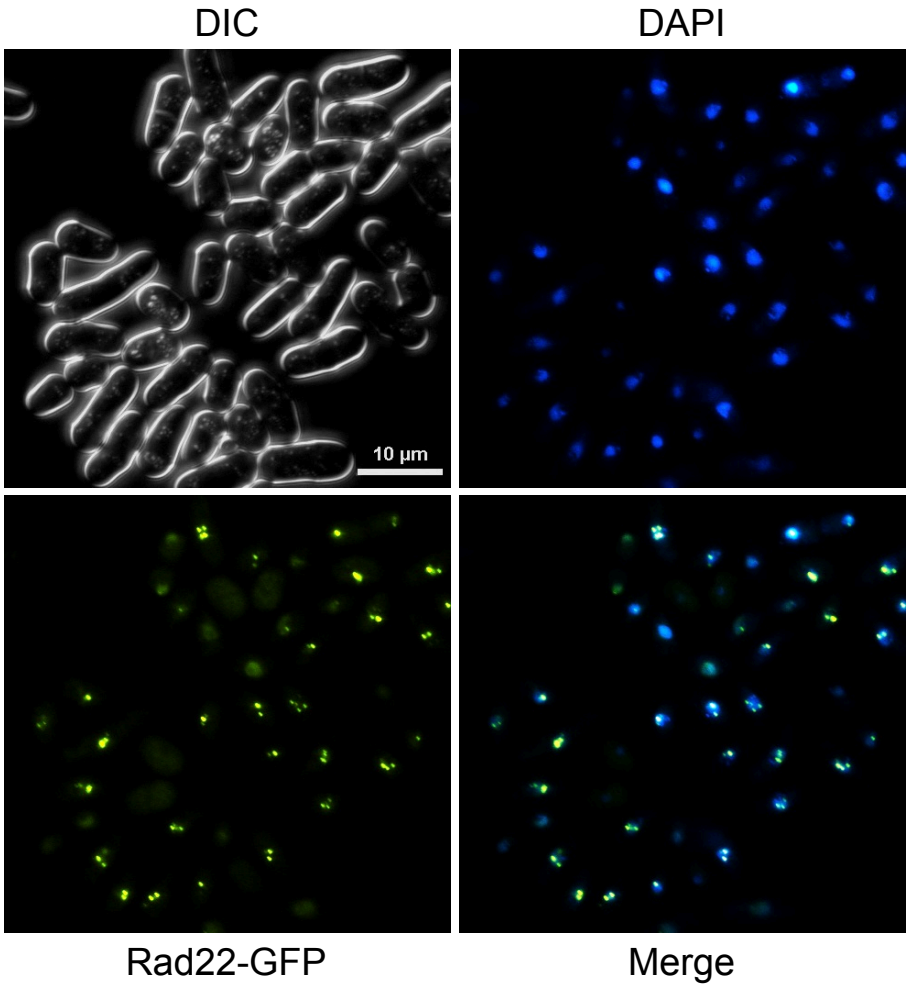
Supplementary Table S1: Fission yeast strains used in this study

Strain		Genotype	Souse
JJ098	h^+	*leu1-32 ura4-D18 spac25b8.16::spac25b8.16-SV40pA-kanMX6*	This study
JJ0910	h^+	*leu1-32 ura4-D18 rad22::rad22-FLATA-kanMX6*	This study
JJ1035	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-abp2-FH6	This study
JJ1036	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-bag101-FH6	This study
JJ1037	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-sap1-FH6	This study
JJ1038	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-ssa1-FH6	This study
JJ1039	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-ssb1-FH6	This study
JJ1040	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-ssb2-FH6	This study
JJ1041	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-rad22-FH6	This study
JJ1042	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-rim1-FH6	This study
JJ1043	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-rti1-FH6	This study
JJ1044	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-rvb2-FH6	This study
JJ1045	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-FH6	This study
JJ1046	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-abp2-FH6	This study
JJ1047	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-bag101-FH6	This study
JJ1048	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-sap1-FH6	This study
JJ1049	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-ssa1-FH6	This study
JJ1050	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-ssb1-FH6	This study
JJ1051	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-ssb2-FH6	This study
JJ1052	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-rad22-FH6	This study
JJ1053	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-rim1-FH6	This study
JJ1054	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-rti1-FH6	This study
JJ1055	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-rvb2-FH6	This study
JJ1056	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-FH6	This study
JJ1103	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-bag101(BAG)-FH6	This study
JJ1104	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus*	This study

		pFOX1-bag101(UBL)-FH6	
JJ1110	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-bag101(BAG)-FH6	This study
JJ1111	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-bag101(UBL)-FH6	This study
JJ1119	h^-	*leu1-32 Dura4::RDUX200(+) bag101D::natMX6*	This study
JJ1121	h^-	*leu1-32 Dura4::RDUX200(+) bag101::bag101-HATA-natMX6*	This study
JJ1125	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-msh2-FH6	This study
JJ1126	h^-	*leu1-32 Dura4::RDUX200(+) rad22-Venus* pFOX1-msh6-FH6	This study
JJ1127	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-msh2-FH6	This study
JJ1128	h^-	*leu1-32 Dura4::RDUX200(+)* pFOX1-msh6-FH6	This study
JJ1129	h^-	*leu1-32 rad22-Venus bag101D::natMX6*	This study
JJ1142	h^-	*leu1-32*	This study
JJ1143	h^-	*leu1-32 bag101D::natMX6*	This study
JJ1144	h^-	*leu1-32* pFOX1-bag101-FH6	This study
mts2	h^-	*leu1-32 mts2-1*	Prof. Matsumoto
mts2+wt	h^-	*leu1-32 mts2-1 mts2$^+$-8myc-LEU2*	Prof. Matsumoto
mts3	h^-	*leu1-32 mts3-1*	Prof. Matsumoto
mts3+wt	h^-	*leu1-32 mts3-1 mts3$^+$-8myc-LEU2*	Prof. Matsumoto

Supplementary Table S2: Plasmids used in this study

Name	Souse
pCtFLATAki-rad22-kanMX6-Trad22	This study
pFOX1-CFH6	This study
pFOX1-abp2-FH6	This study
pFOX1-bag101-FH6	This study
pFOX1-sap1-FH6	This study
pFOX1-ssa1-FH6	This study
pFOX1-ssb1-FH6	This study
pFOX1-ssb2-FH6	This study
pFOX1-rad22-FH6	This study
pFOX1-rim1-FH6	This study
pFOX1-rti1-FH6	This study
pFOX1-rvb2-FH6	This study
pFOX1-msh2-FH6	This study
pFOX1-msh6-FH6	This study
pFOX1-bag101(BAG)-FH6	This study
pFOX1-bag101(UBL)-FH6	This study
pCtHATAki-Pbag1-natMX6-Tbag1	This study
pCtHATAki-bag101-kanMX6-Trad22	This study

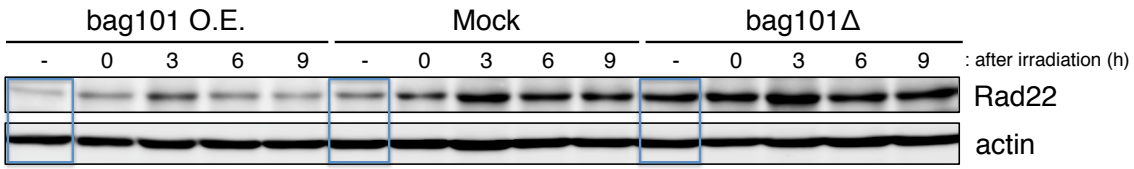


Supplementary Figure S1: Rad22-foci formation. Rad22 foci formation was observed with a Nikon Eclipse 90i fluorescent microscope. The cells containing at least one of Rad22-foci were counted for Rad22-foci positive cells.

Supplementary Table S3: Primers used for cloning, construction of Bag101 domain-deleted mutants and quantitative PCR

Name	Sequence (5'-3')
Sal-abp2-F	GGGGGTCGACTATG-AACTTTTATTCCTTGTTGCCATCTAGACATG
Nde-abp2-R	GGGGGGGGCATATG-GTCAAACACTGCATTCATAATCTTTCTC
Sal-bag101-F	GGGGGTCGACTATG-TCAGAAAAGACTAGCACAGTTACAATACAC
Nde-bag101-R	GGGGGGGGCATATG-TGCGGCCACTTCTTGGCTTGTTTG
Sma-sap1-F	GGGCCCGGGTTATG-GAAGCTCCCAAGATGGAACTG
Nde-sap1-R	GGGGGGGGCATATG-ATGGTCACCAAGATTAGGAGAGATG
Sal-ssa1-F	GGGGGTCGACT-ATGAGCAAGTCTATCGGTATTGATTTG
Nde-ssa1-R	GGGGGGGGCATATG-ATCCACTTCTTCAACCTCAGGTC
Sal-ssb1-F	GGGGGTCGACT-ATGGCTGAGCGATTATCCGTGGG
Nde-ssb1-R	GGGGGGGGCATATG-TTGAGCAGACTCAATGAAATTTATC
Sal-ssb2-F	GGGGGTCGACT-ATGGCTTATGATGCTTTTGGCAAGC
Nde-ssb2-R	GGGGGGGGCATATG-TTGGTCTTGTAAAACACTTTTAAAATGATT
Sal-rad22-F	GGGGGTCGACT-ATGTCTTTTGAGCAAAAACAGC
Nde-rad22-R	GGGGGGGGCATATG-TCCTTTTTTGGCTTTCTTATCCAC
Sal-rim1-F	GGGGGTCGACTATG-CTATTCTTAAAATCCAGCAGAGCATTTTC
Nde-rim1-R	GGGGGGGGCATATG-GAAGCTGTTAATCATAGGATCTGCG
Sal-rti1-F	GGGGGTCGACT-ATGGGCTCGCTACCTGATCAATC
Nde-rti1-R	GGGGGGGGCATATG-TTTCGTTGAGAACGTGTTTGCAGAG
Sal-rvb2-F	GGGGGTCGACTATG-TCGATTTCGGTGACTTCTCATAATGATG
Nde-rvb2-R	GGGGGGGGCATATG-ATCTTCCTGCATTGCAACTGCATTATC
Sma-msh2-F	GGGCCCGGGTTATG-TCTTCTAGAAACGCTTCAATTGCTAATG
Sfo-msh2-R	GGGGGGGGGGCGCC-TGAGGAAACCTCATTTGTGGATAC
Sma-msh6-F	GGGCCCGGGTTATG-AGCGTTGGGAACGTTGGCAAG
Sfo-msh6-R	GGGGGGGGGGCGCC-TTCAAACGAATCAAGTATGAGAGGTATATTC
i_bag101(BAG)-R	ATTCTCCGCTTCCGCTTTCGGAG
i_bag101(UBL)-F	CCTGTATTTTCGCGTATTTCTGG
BsiWI-Pbag1-F	GGGGGGCGTACG-ATATGATCCTTACCCAATTCCGTG

Acc65I-Pbag1-R	GGGGGGGGTACC-TGACGATATTAAGTCGACTTGCTTAAC
Spe-Tbag1-F	GGGGGGACTAGT-TAGGCTTTATTATTTTTATAGACGTTACC
Nhe-Tbag1-R	GGGGGCTAGC-GGACGTGCAATAACTGTAGAGCG
Sac-bag101-R	GGGGGAGCTC-TGCGGCCACTTCTTGGCTTGTTTG
rt_hxk2_730+	GGTGATCATATGCTTATCAACATGG
rt_hxk2_846-	AAAGATTTGACGACCAGCATTGG
rt_rad22_1059+	TCCCTTCACGAACAACTTTAATCC
rt_rad22_1177-	GCTTAATTATAGGTAAAACCGATGCC



Supplementary Figure S2: levels of Rad22 protein expressed in bag101 mutants.

The amounts of Rad22 in cells overexpressing bag101 or deleting bag101 were determined by Western blotting with anti-Rad22 antibodies at the indicated time after irradiation. The Rad22 protein levels in no-irradiated cells (indicated by blue rectangles) were represented in Figure 3a.