Supplemental Data

Rif1 and Rif2 inhibit localization of Tel1 to DNA ends

Y. Hirano, K. Fukunaga and K. Sugimoto

I. Supplemental Experimental Procedures

Strains

The TG_{162}-HO cassette was constructed from the TG_{81}-HO cassette (Hirano and Sugimoto, 2007). An *Mfe*I site was introduced into the TG_{81} sequence by PCR amplification. The resulting PCR fragment was digested with *Mfe*I and *Not*I, and cloned into the *Eco*RI/*Not*I sites of pTG-HO (Hirano and Sugimoto, 2007), creating pTG_{162}-HO. The $TetO_2$, $TetO_4$, or $TetO_8$ construct was obtained as follows. First, two oligonucleotides were annealed to generate a $TetO_2$ array containing two copies of the 19 bp *tetO* promoter sequence (Dingermann et al., 1992). $TetO_2$ was first cloned into the *Eco*RI/*Xho*I sites of pSD155 (Diede and Gottschling, 1999, 2001), generating $ptetO_2$. To duplicate the $TetO_2$ sequence, the *Mfe*I-*Not*I fragment of $ptetO_2$ was cloned into *Eco*RI/*Not*I-treated $ptetO_2$, generating $ptetO_4$. Likewise, $ptetO_8$was obtained using $ptetO_4$. These plasmids were treated with *Eco*RI and *Sal*I. The resulting *Eco*RI-*Sal*I fragments containing the $TetO_2$, $TetO_4$ or $TetO_8$ sequence were cloned into *Mfe*I/*Sal*I-treated pHO plasmid (Hirano and Sugimoto, 2007), generating $ptetO_2$-HO, $ptetO_4$-HO or $ptetO_8$-HO, respectively. Oligonucleotides containing four copies of *lacO* sequence (Lewis, 2005), were used to generate the $placO_4$ plasmid containing the $LacO_4$ sequence. To duplicate the $LacO_4$ sequence, the *Mfe*I-*Not*I fragment of $placO_4$ was cloned into *Eco*RI/*Not*I-treated $placO_4$, generating $placO_8$. As the ptetO8 was constructed above, $placO_{16}$ was obtained using $placO_8$. The *Mfe*I-*Not*I fragment containing the TG_{81} sequence was cloned into *Eco*RI/*Not*I-treated $pLacO_{16}$-HO, generating $pLacO_{16}$-TG_{81}-HO. All the above plasmids were digested with *Not*I and *Sal*I to integrate the cassettes into the *MAT*a-inc strain (Nakada et al., 2003a). Cells containing the $LacO_{16}$-TG_{81}-HO cassette were obtained by using the $pLacO_{16}$-TG_{81}-HO plasmid. Cells containing the TG_{162}-HO, $tetO_2$-HO, $tetO_4$-HO, $tetO_8$-HO or $LacO_{16}$-TG_{81}-HO cassette were generated by the plasmid pTG_{162}-HO, $ptetO_2$-HO, $ptetO_4$-HO, $ptetO_8$-HO or $pLacO_{16}$-TG_{81}-HO, respectively. Epitope tagging of *RAP1*, *RIF1* and *RIF2*

was performed by a PCR-based strategy (Hirano and Sugimoto, 2006; Knop et al., 1999). Cells carrying a *rif1Δ*, *rif2Δ*, *sir2Δ* or *tel1Δ* mutation were obtained by transformation with PCR fragments marked with *LEU2* (Wakayama et al., 2001), *URA3* (Reid et al., 2002) or *HphMX4* (Goldstein and McCusker, 1999). Precise integration of each construct was confirmed by PCR. Other mutations were described previously (Hirano and Sugimoto, 2007; Nakada et al., 2003a; Nakada et al., 2003b; Wakayama et al., 2001).

Plasmids

To obtain the tetR-fusion gene constructs, the *tetR* coding sequence was amplified by PCR from pCM224 (Belli et al., 1998), adding the SV40 nuclear localization sequence (CTPPKKKRKVA) at its C-terminus. The PCR fragment was digested with *Sal*I and *Sac*I, and cloned into the *Sal*I/*Sac*I sites of YCplac33 (Gietz and Sugino, 1988) carrying a truncated version of the *ADH1* promoter, generating ptetR. The *Sac*II-*Sac*I fragments containing the *RIF1*, *RIF2* or *RAP1* coding sequence were cloned into *Sac*II-*Sac*I treated ptetR, resulting in ptetR-RIF1, ptetR-RIF2 or ptetR-RAP1, respectively. The *Sac*II-*Sac*I fragments containing the HA-epitope fused *RIF1* or *RIF2* coding sequence were cloned into *Sac*II/*Sac*I-treated ptetR, resulting in ptetR-RIF1-HA or ptetR-RIF2-HA, respectively. The *lacI* coding sequence with the SV40 nuclear localization signal was amplified from pAFS78 (Straight et al., 1996) fusing an FLAG epitope at the C-terminus. The PCR fragment was digested with *Sal*I and *SacI*, and cloned into the *Sal*I/*Sac*I sites of YCplac33 carrying a truncated version of the *ADH1* promoter, generating pLacI-FLAG. Fragments containing the *GAL10* promoter or encoding an N-terminal Tel1 region were amplified by PCR, adding one FLAG epitope to each fragment, and digested with *Not*I and *Ngo*MIV or *Ngo*MIV and *Rsr*II, respectively. These fragments were cloned into *Not*I/*Rsr*II-treated pDM196 (Morrow et al., 1995), generating pGAL-FLAG-TEL1. To create pGST-XRS2C, the C-terminus of Xrs2 (amino acids 474-854) was amplified by PCR and cloned into pGEX-6P-1 (GE Healthcare). To express His-Rif2-FLAG proteins, the coding sequence of *RIF2* was amplified by PCR, adding the sequence encoding two FLAG epitopes at the C-terminus. The PCR fragment was digested with *Eco*RI and *Sac*I, and cloned into pET28a (Novagen), generating pET-RIF2-FLAG. Fragments containing an N-terminal (amino acids 1-170), a central (amino

acids 91-250), or C-terminal (amino acids 171-395) region of Rif2 were obtained after PCR amplification and cloned into pET28a, creating pET-RIF2(1-170), pET-RIF2(91-250) or pET-RIF2(171-395). pET28a contains the sequences encoding His- and T7-tags. The same *RIF2* PCR fragments and a fragment encoding three myc epitopes were cloned into ptetR, resulting in ptetR-RIF2(1-170), ptetR-RIF2(91-250) or ptetR-RIF2(171-395). YCpA-GAL-HO was previously described (Nakada et al., 2003a). The pYtel plasmid containing a telomeric TG repeat was obtained from A. Matsuura.

PCR primer for ChIP assay

The sequences of primers for the HO set were 5'-GTTGTTTCTGAAACATGGCAAAGG-3' and 5'-CAACCAAACCGTTATTCATTCGTG-3', and those for the *SMC2* locus were 5'- AAAGACTGAGCTGAATGAAGTCTC -3' and 5'-CGTCTGAGAATCTTGAACAACAAC -3'. The primers for the telomere VL-R and XV-L were synthesized as described previously (Fisher et al., 2004; Hector et al., 2007).

Protein purification

FLAG-Tel1 proteins were purified from budding yeast cells. Cells carrying pGAL-FLAG-TEL1 were cultured in medium containing 2% galactose for 5 h. Cells were suspended in the lysis buffer (20 mM Tris-HCl at pH 8.0, 0.1% Triton X-100, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM Na_3VO_4, 40 mM β-glycerophosphate, 15 mM ρ-NO_2-phenylphosphate, 1 μg/ml of leupeptin, 1 μg/ml of pepstatin, 0.5% aporotinin, 1 mM PMSF) and physically disrupted with glass beads. After centrifugation, supernatants were incubated with anti-FLAG M2 affinity gel (Sigma) at 4°C for 2 h. The beads were washed three times with the lysis buffer and bound proteins were eluted with the elution buffer (20 mM Tris-HCl at pH 8.0, 0.01% Triton X-100, 100 mM NaCl) containing 150 μg/ml of 3xFLAG peptides (Sigma). GST or GST-Xrs2C proteins were prepared from *E. coli* carrying pGEX-6P-1 or pGST-XRS2C, respectively. After induction with IPTG for 3 hr, GST proteins were purified using glutathione Sepharose according to the manufacturer's instructions (GE healthcare). His-Rif2-FLAG or His-Rif2 fragments were purified from *E. coli*. Cells transformed with pET-RIF2-FLAG, pET-RIF2 (1-170), pET-RIF2 (91-250) or pET-RIF2 (171-395) were cultured at 19°C for 14 hr in the presence of IPTG. Proteins were purified on a Ni-column (Novagen) as suggested by the

manufacturer. FLAG-tagged proteins were further purified on anti-FLAG M2 affinity gel as above.

II. Supplemental Figures

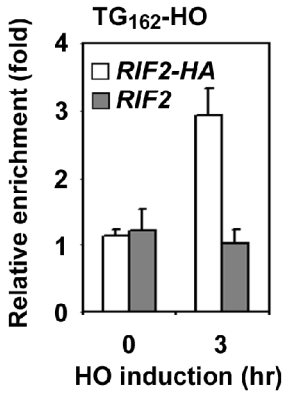

Fig. 1S. Association of Rif2 with TG_{162} ends. TG_{162}-HO cells expressing HA-tagged or non-tagged Rif2 proteins were analyzed by ChIP assay as in Fig. 1C.

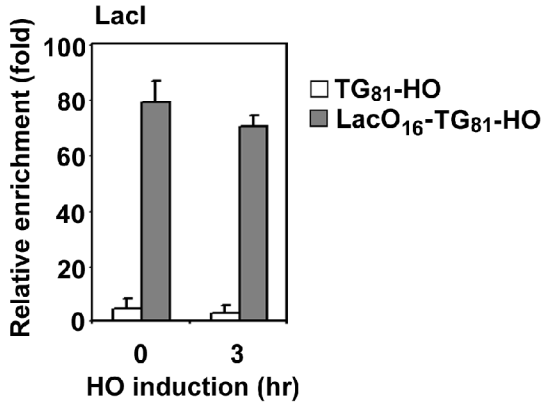

Fig. 2S. Association of LacI with the LacO array near a HO-induced break. TG_{81}-HO cells or $LacO_{16}$-TG_{81}-HO cells expressing FLAG-tagged LacI proteins were analyzed by ChIP assay as in Fig. 1C.

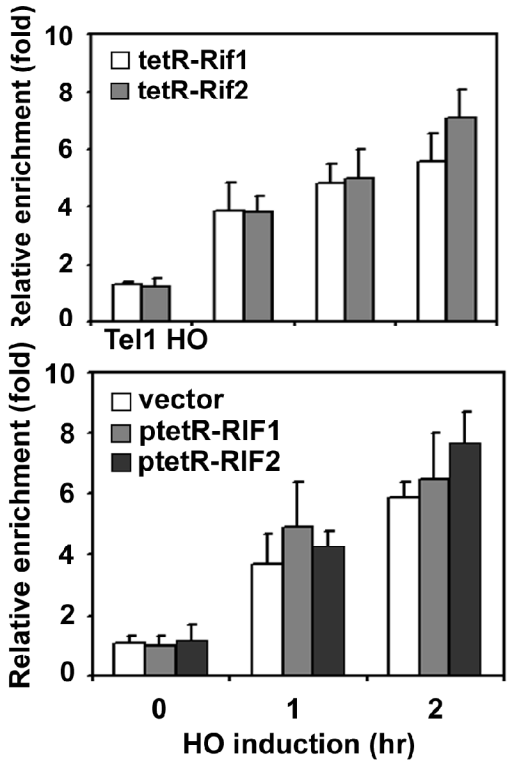

Fig. 3S. Association of TetR-Rif1 and TetR-Rif2 with various TetO repeats. HO, $TetO_2$-HO, $TetO_4$-HO and $TetO_8$-HO cells expressing tetR-Rif1-HA or tetR-Rif2-HA proteins were grown in sucrose, harvested without *HO* induction and analyzed by ChIP assay as in Fig. 1C. HO cells contain no TetO repeat.

Fig. 4S. Association of Tel1 with DSBs without the TetO array in cells expressing

TetR-Rif1 or TetR-Rif2.
HO cells expressing Tel1-HA were transformed with ptetR-RIF1, ptetR-RIF2 or the control vector, together with the GAL-HO plasmid, and analyzed by ChIP assay as in Fig. 1C. Strains used contain a *rap1-ΔC* mutation.

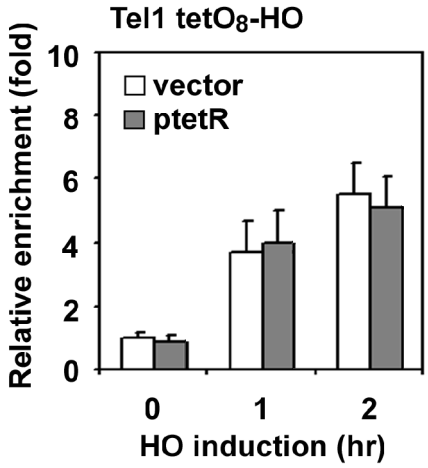

Fig. 5S. Association of Tel1 with DSBs near the $TetO_8$ array in cells expressing TetR by itself.
$TetO_8$-HO cells expressing Tel1-HA were transformed with ptetR or the control vector, together with the GAL-HO plasmid, and analyzed by ChIP assay as in Fig. 1C. Strains used contain a *rap1-ΔC* mutation.

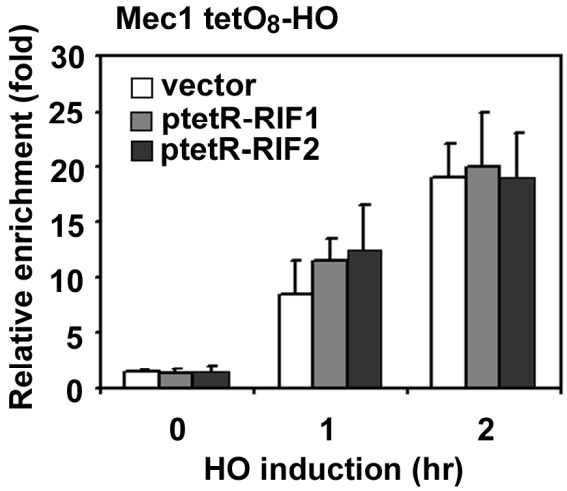

Fig. 6S. Association of Mec1 with DSBs near the $TetO_8$ array in cells expressing TetR-Rif1 or TetR-Rif2.
$TetO_8$-HO cells expressing Mec1-HA were transformed with ptetR-RIF1, ptetR-RIF2 or the control vector, together with the GAL-HO plasmid, and analyzed by ChIP assay as in Fig. 1C. Strains used contain a *rap1-ΔC* mutation.

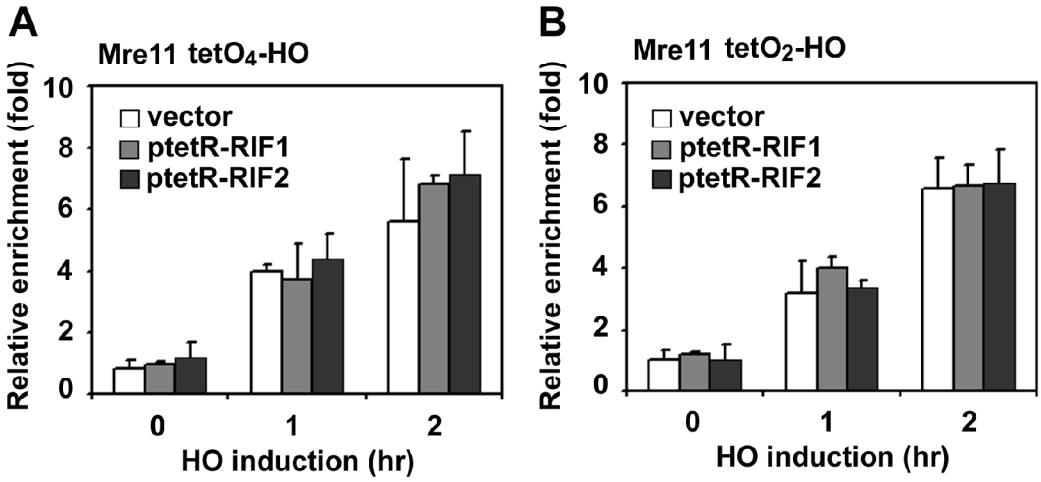

Fig. 7S. Mre11 association with $TetO_4$ or $TetO_2$ ends in cells expressing TetR-Rif1 or TetR-Rif2 proteins.
$TetO_4$-HO (A) or $TetO_2$-HO (B) expressing Mre11-myc were transformed with ptetR-RIF1, tetR-RIF2 or the control vector, together with the GAL-HO plasmid, and analyzed by ChIP assay as in Fig. 1C. Strains used contain a *rap1-ΔC* mutation.

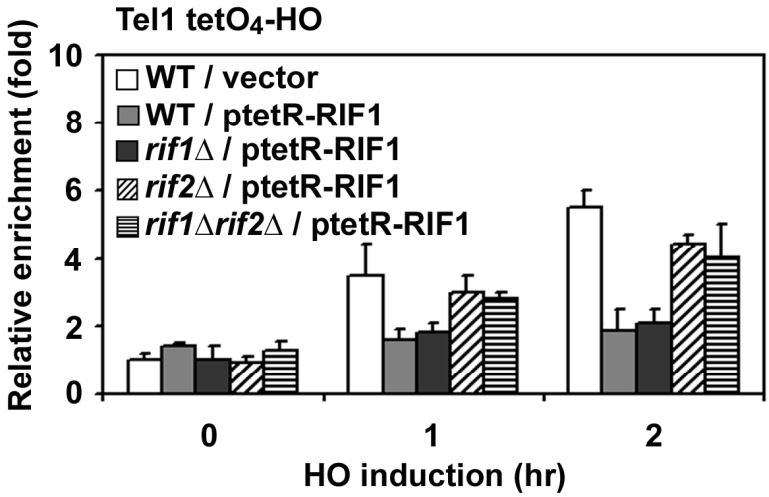

Fig. 8S. Effect of endogenous *RIF1* gene on TetR-Rif1-mediated inhibition of Tel1 binding at $TetO_4$ ends.
$TetO_4$-HO cells expressing Tel1-HA were transformed with ptetR-RIF1 or the control vector, together with the GAL-HO plasmid, and analyzed by ChIP assay as in Fig. 1C. Strains used were wild-type, *rif1Δ* single, *rif2Δ* single or *rif1Δ rif2Δ* double mutant cells.

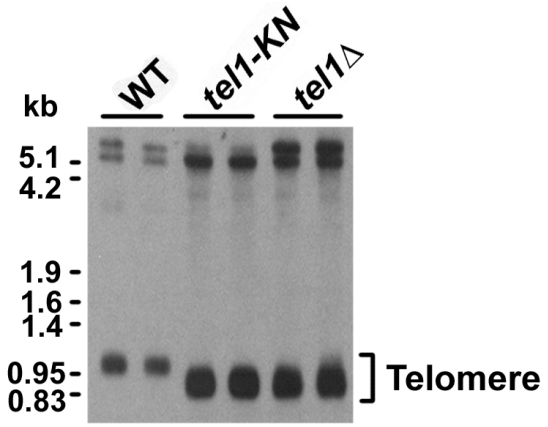

Fig. 9S. Effect of *tel1-KN* or *tel1Δ* mutation on the length of endogenous telomeres.
Genomic DNA was prepared from cells and digested by XhoI, and analyzed by Southern blot using the EcoRV-XhoI fragment from the pYtel plasmid as a probe. Two independent colonies of wild-type, *tel1-KN* and *tel1Δ* mutants used in Fig. 4E were analyzed.

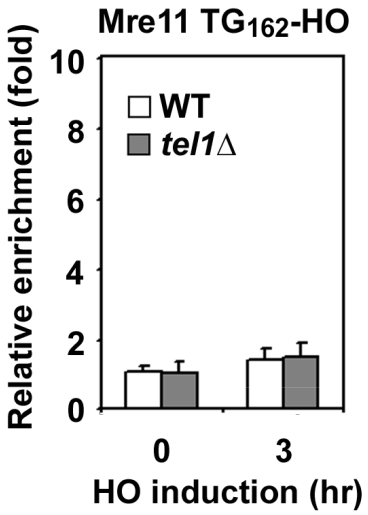

Fig. 10S. Effect of *tel1Δ* on association of Mre11 with TG_{162} ends.
TG_{162}-HO cells expressing Mre11-myc were analyzed by ChIP assay as in Fig. 1C.

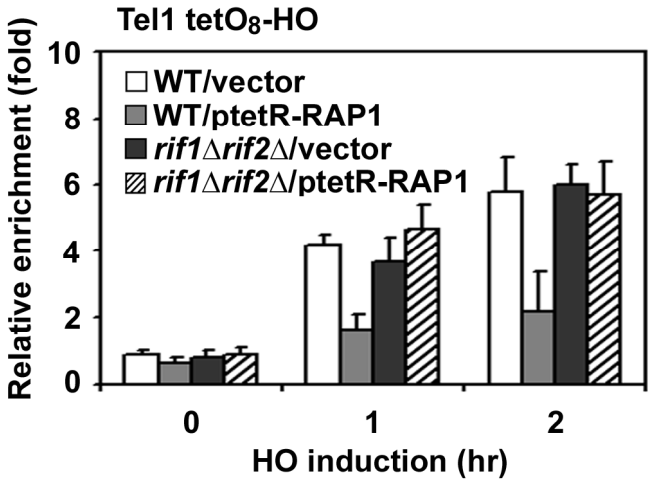

Fig. 11S. Effect of TetR-Rap1 expression on Tel1 association in *rif1Δ rif2Δ* cells. $TetO_8$-HO or $TetO_8$-HO *rif1Δ rif2Δ* cells expressing Tel1-HA were transformed with ptetR-RAP1 or the control vector, together with the GAL-HO plasmid, and analyzed as in Fig. 1C to monitor Tel1 association. The strains used here contain a *sir2Δ* mutation to rescue defective HO cleavage.

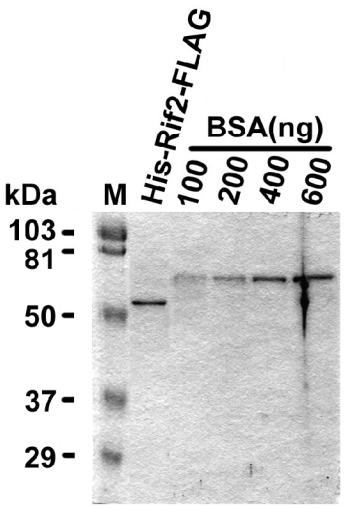

Fig. 12S. Purification of His-Rif2-FLAG protein from *E. coli*.
Purified His-Rif2-FLAG proteins were separated on 12% SDS-PAGE and stained win Coomassie Brilliant Blue. The left lane contains protein molecular-weight standard (M). BSA was loaded to estimate protein concentration.

III. References

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