Supplemental Material for "Co-localization of Mec1 and Mrc1 is sufficient for Rad53 phosphorylation *in vivo*"

Supplemental Methods

Rad53 in situ *kinase assay (ISA)*

ISAs were performed according to the protocol of Pellicioli *et al*. (1999). Cell pellets were TCA-precipitated, run on an SDS-PAGE gel, and transferred to a PVDF membrane in buffer containing methanol. Membranes were rinsed briefly with TBST, denatured for one hour at room temperature in 7M guanidine-HCL/50 mM dithiothreitol (DTT)/2 mM EDTA/50 mM Tris-HCl pH 8.0, washed twice with 1X TBS, and then renatured overnight at 4°C with four changes of buffer in 2 mM DTT/2 mM EDTA/0.04% Tween-20/10mM Tris-HCl pH 7.5/140 mM NaCl/1% BSA. Membranes were then incubated at room temperature for 1 hour in 30 mM Tris-HCl, pH 7.5 before pre-equilibration in kinase buffer (1mM DTT, 0.1 mM EGTA, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 40 mM Hepes-NaOH pH 8.0, 0.1 mM sodium orthovanadate) for 30 minutes at room temperature. Kinase assays were then performed in fresh kinase buffer in the presence of 100μCi per membrane [γ-^{32}P]ATP for one hour at room temperature. Membranes were washed for 10 minutes each in 30 mM Tris-HCl pH 7.5, 30 mM Tris-HCl pH 7.5, 30 mM Tris-HCl pH 7.5/0.1% NP-40, 30 mM Tris-HCl pH 7.5, 1M KOH, water, 10% TCA, and water, and then dried and exposed to a PhophoImager.

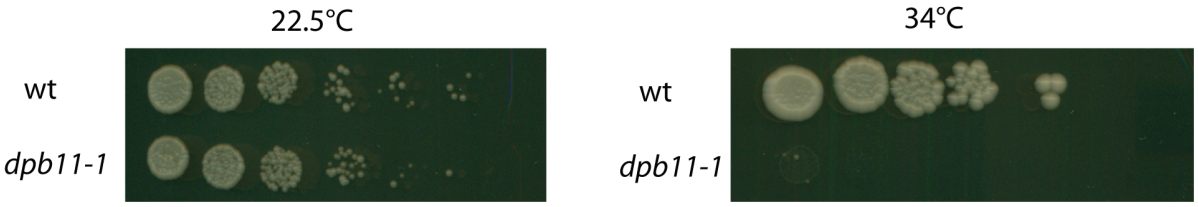
Rad53 activation was quantified by measuring the intensity of the Rad53 band and of the background band that serves as a loading control, and subtracting the background of the blank membrane from each. Then, the corrected intensity of the Rad53 band was divided by the corrected intensity of the loading control to give the relative Rad53 intensity. The highest

intensity was set to 1. To give the Rad53 activation for each strain, the relative Rad53 intensity of the untreated sample was subtracted from the relative Rad53 intensity of the HU- or MMS-treated sample.

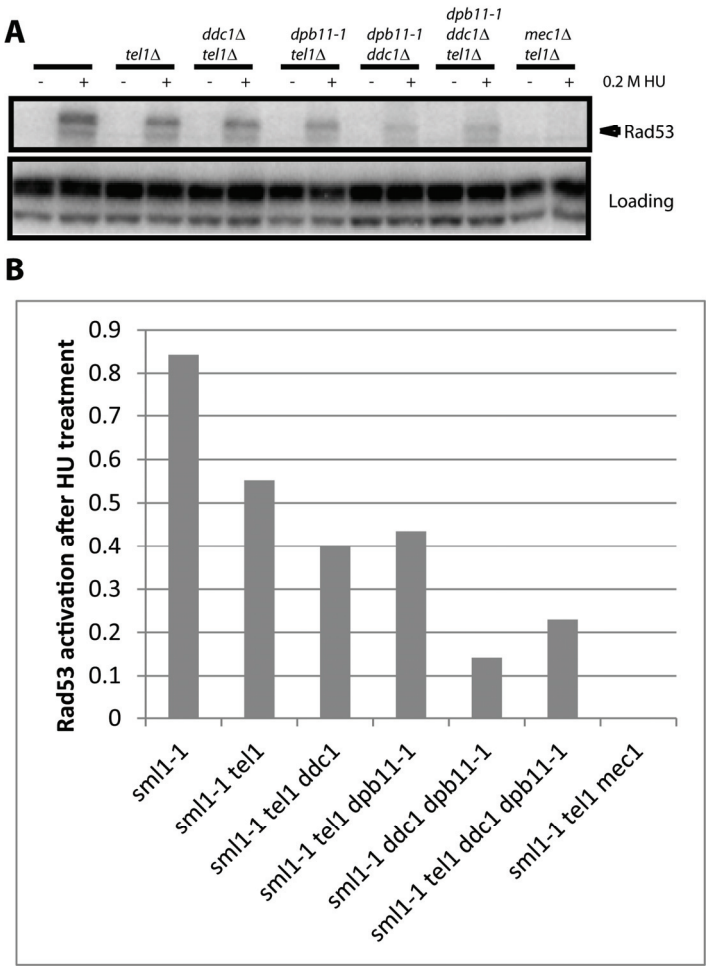
Supplemental References

Pellicioli, A., Lucca, C., Liberi, G., Marini, F., Lopes, M., Plevani, P., Romano, A., Di Fiore, P.P., Foiani, M. (1999). Activation of Rad53 kinase in response to DNA damage and its effect in modulating phosphorylation of the lagging strand DNA polymerase. EMBO J. *18(22)*: 6581-72.

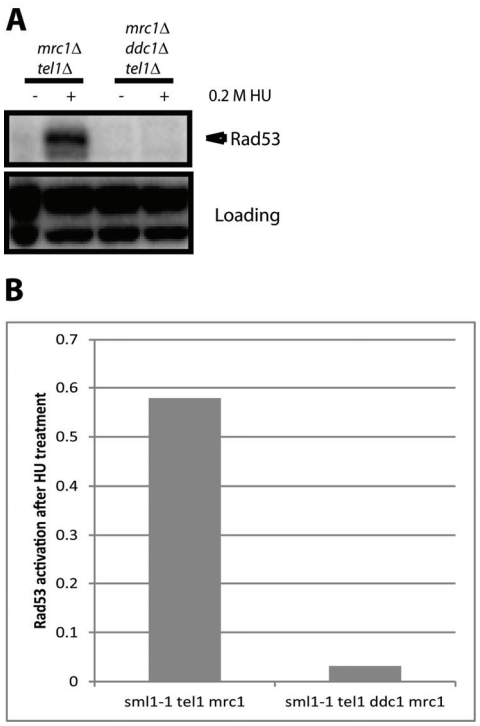
Figure Legends



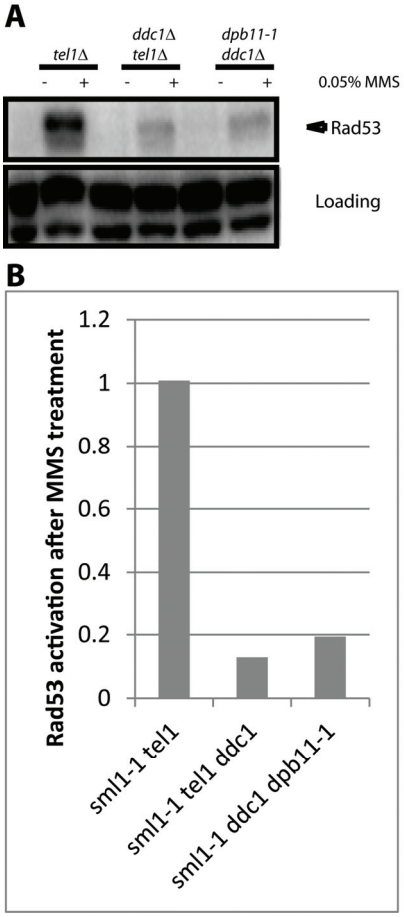
Supplementary Figure S1. *dpb11-1* cells cannot grow at 34°C



Supplementary Figure S2 (related to Figure 3). Rad53 can be activated in response to replication stress in the absence of 9-1-1 and Dpb11. (A) Strains with the indicated genotype were grown asynchronously and then treated with 0.2 M hydroxyurea (HU) for 4 hours at 22.5°C. All strains carry the *sml1-1* mutation, which suppresses lethality of a *mec1*Δ. Rad53 activation was assayed by ISA. (B) Rad53 activation was quantified as described in the Supplemental Methods.



Supplementary Figure S3 (related to Figure 4A). Rad53 phosphorylation in the absence of 9-1-1 requires Mrc1. (A) Strains with the indicated genotype were grown asynchronously and then treated with 0.2 M HU for 4 hours at 22.5°C. All strains carry the *sml1-1* mutation, which suppresses lethality of a *mec1*Δ. Rad53 activation was assayed by ISA. (B) Rad53 activation was quantified as described in the Supplemental Methods.



Supplementary Figure S4 (related to Figure 4D). Rad53 activation in response to MMS is equivalent in *ddc1*Δ *tel1*Δ and *ddc1*Δ *dpb11-1* mutants. (A) Strains with the indicated genotype were grown asynchronously and then treated with 0.05% methyl methanesulfonate (MMS) for 4 hours at 22.5°C. All strains carry the *sml1-1* mutation, which suppresses lethality of a *mec1*Δ. Rad53 activation was assayed by ISA. (B) Rad53 activation was quantified as described in the Supplemental Methods.