

Supplementary Legends:

Figure S1. A: Close up view of AMPPNP and Mg^{2+} coordination within the Rad50 ATPase active site. AMPPNP is marked as color coded sticks; the final $2F_o-F_c$ density around AMPPNP is contoured at 1.0σ and colored in dark blue. Walker A and signature motifs coordinate the phosphate moieties in the AMPPNP molecule. Mg^{2+} is coordinated by two water molecules, oxygen of phosphate moieties in AMPPNP and side chains of the Walker A motif and Q-loop (Q142).

B: Depicted are two adjacent symmetry related molecules indicating the quasi-continuous DNA double strand in the crystal lattice. The final $2F_o-F_c$ density around DNA is contoured at 0.8σ and marked in dark blue.

Figure S2. Structural comparison of the tmRad50^{NBD}-Mre11^{HLH} apo (PDB code: 3QF7) and DNA-bound structures. Both models show almost identical architecture. The most pronounced repositioning is visible at the coil-coil domains and the HLH motifs.

Figure S3. AMPPNP-induced tmRad50^{NBD}-Mre11^{HLH} dimerization.

A Left panel: wild-type tmRad50^{NBD}-Mre11^{HLH} complex remains in a monomeric form in the absence of AMPPNP and Mg^{2+} (dotted line) and forms a stable dimer upon AMPPNP addition (solid line); A right panel: ATP fails to form a stable tmRad50^{NBD}-Mre11^{HLH} dimer.

B: Point mutations do not impair tmRad50^{NBD}-Mre11^{HLH} dimerization and dimer stability except from the signature motif mutant S768R.

Figure S4: Quantitation (bar graphs) of the residual unbound DNA for different tmRad50^{NBD}-Mre11^{HLH} mutants (see Fig. 3). The estimated half-maximal value is depicted in Fig. 3 to assess the approximate binding strength of different mutant proteins.

Figure S5. A: DNA binding activity of the wild-type tmRad50^{NBD}-Mre11^{HLH} complex with 30mer or 60mer dsDNA. Binding efficiency is significantly increased with the longer DNA species.

B: Table of mutations analysed in yeast *in vivo* assays and the corresponding residue in *Thermotoga maritima* shown together with the position of the relevant residues in the tmRad50 DNA structure.

Figure S6. A Rad50 expression levels in *S. cerevisiae* Rad50 mutants. Western blot analysis performed on yeast lysate show comparable levels of Rad50 in wild-type and mutated *S. cerevisiae*.

B: In vivo plasmid repair assay of the yeast mutant strains Rad50 K110E, R125E and Δ Rad50. Whereas the Δ Rad50 strain lead to dramatically reduced transformant yields for NcoI-linearized pRS315-Kan plasmids, the Rad50 point mutants showed only minor changes in plasmid rescue efficiency which are negligible considering the standard error of the experiment. For this experiment competent cells for yeast strains of the indicated genotype were transformed with either supercoiled pRS315-Kan or NcoI linearized pRS315-Kan plasmids. For each strain the value plotted is the number of transformants obtained with NcoI linearized vector relative to the number obtained with supercoiled vector. Thereby the number of transformants obtained with the Δ Rad50 strain rescued with the pRS313 Rad50 wild type plasmid has been normalized to 1.