Figure EV1. The central groove at the interface between Rad50 dimer.
A A surface diagram of the central groove in Rad50 dimer. The surface was sliced down the middle to show the groove architecture. At the edges of Rad50 dimer, which are located at the N-terminal region (or lobe I, residues 1–137 and 939–1,005), the groove is 5 Å deep, but the depth increases to 13 Å as it approaches the middle of the groove. Because a linear dsDNA interacts with both edges of Rad50 dimer, a large empty space is formed between the DNA and the middle of a central groove of a Rad50 dimer. The dimensions of the groove are indicated.
B Diagram of the ATP–Rad50 dimer–DNA complex presented as an electrostatic color-coded surface representation. The positively charged surface is shown in blue, and the negatively charged surface is shown in red.

Figure EV2. Comparison of the structures between ATPγS–Mre11/Rad50–DNA complex.
A Structure of the DNA-bound ATPγS–Mre11/Rad50–DNA complex is shown in two different views. ATP molecule is shown in spheres. DNA-binding residues are shown in red spheres, and only a few residues are labeled for clarity.
B Structure of the AMP–PNP–TmRAD50–DNA complex is shown in two different views. DNA is bound between the coiled-coil region and lobe I (strand-loop-helix) in one NBD. Mre11 C-terminal peptide is shown in pink, and Rad50 is shown in gray. DNA-binding residues are shown as red spheres. Orientation is same as in (A).
**Figure EV3. DNA binding activity of the MjMRcd or TmMR complex toward a closed circular dsDNA.**

A, B DNA binding analysis for the interactions between WT or four MjMRcd mutants and a linear dsDNA in the absence (A) or presence (B) of AMP-PNP/Mg$^{2+}$. A linear DNA was prepared by cleaving pCDFDuet-1 plasmid using EcoRI. Each protein sample was incubated with a linear dsDNA (3.5 nM) for 30 min on 4°C. The molar ratio of protein:DNA is as following: lane 2, 50:1; lane 3, 100:1; lane 4, 200:1; lane 5, 500:1; lane 6, 1,000:1; and lane 7, 2,000:1.

C, D Interactions of the WT or five full-length TmMR mutants with a linear DNA were examined in the absence (C) or presence of AMP-PNP/Mg$^{2+}$ (D). Reaction conditions are as in (A, B).

E, F DNA binding analysis for the interactions between WT or five full-length TmMR mutants and a 15-nt bubble DNA (2.5 nM) in the absence (E) or presence (F) of AMP-PNP. Reaction conditions are as in (A, B). The molar ratio of protein:DNA is as following: lane 2, 100:1; lane 3, 200:1; lane 4, 500:1; lane 5, 1,000:1; and lane 6, 2,000:1. We note that some (for WT TmMR) or most (for TmMR Δ54–56) fraction of the complex-bound DNA is stacked on the wall. This may be partly due to the aggregation upon protein–DNA association in the wall or formation of a second complex.

Source data are available online for this figure.
Figure EV4. Conformational change of DNA upon binding to the ATPγS–MjMR complex.
A  Superposition of the structures of B-form DNA (gray) and ATPγS–MjMR-bound DNA (orange/yellow). Bases are numbered as in Fig 1B.
B  Top: Structure of the ATPγS–MR-bound DNA (orange, yellow). A cartoon for the MjMR complex is shown as green and blue spheres at the minor groove of the DNA bound to ATPγS–MR. The view is the same as in the top figure. The distances between the phosphates are shown, and those from ideal B-form DNA are shown inside parenthesis. Bottom: Structure of the B-form DNA is shown in the same orientation as above.
Figure EV5. Comparison of the structures between apo- and DNA-bound MjMR complexes.

A, B Close-up view of the local structures showing the movement of helices α6 and α9 of Rad50 upon binding of DNA. Local structure of the MjMR complex in the absence (A) or presence (B) of DNA. In the presence of DNA (B, green), Leu155 and Leu156 shift toward the hydrophobic pocket formed by Phe140, Phe899, Ile931, and Leu935.

C, D Close-up view showing the local structural changes in the absence (C) or presence (D) of DNA. In the presence of DNA, an ion pair (D122-K154) is broken, whereas a new ion-pair network (D159, K163, R939) is formed and interacts with the main chain of G120 to provide the stability of the rotated coiled-coil.

E Local conformation change of the α1–α2 loop upon binding to DNA. Alignment was done using their NBDs, and structures are displayed in the same manner as in Fig 4B.