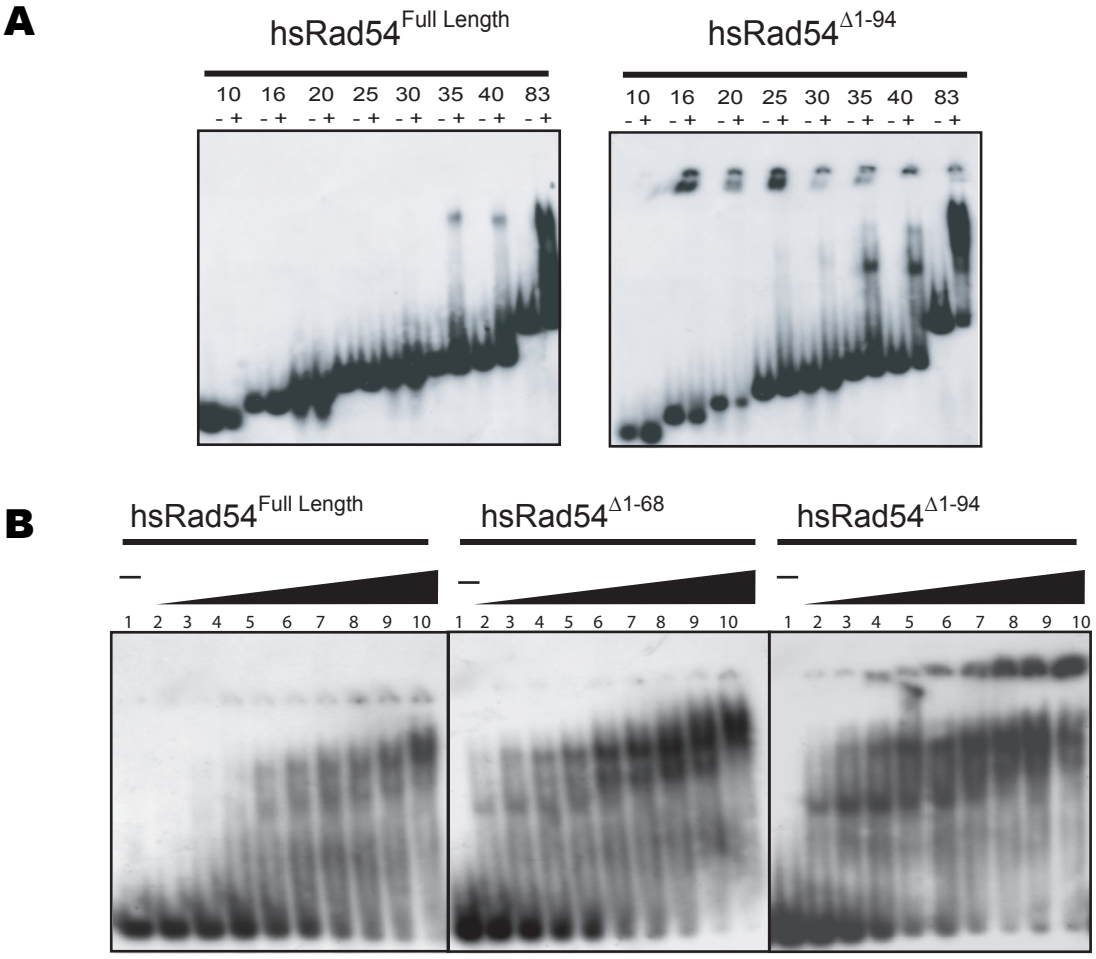
Supplementary Figure 4 DNA binding activity of Rad54.



Oligonucleotide length preference. Full-length hsRad54 at 64nM (A) and hsRad54Δ1-94 (B) at 32 nM were incubated with 1nM of increasing lengths of double stranded radiolabeled oligomer (10, 16, 20, 25, 30, 35, 40, 83 bp). Reactions were analyzed by 4.8% PAGE electrophoresis. Influence of the protein length on DNA binding. Increasing amounts of hsRad54full length (C), hsRad54Δ1-68 (D) hsRad54Δ1-94 (E) protein (lane 1-10: 0, 7.5, 15.5, 31, 62, 125, 250, 500, 1000, 2000 nM) were incubated with 1 nM radiolabeled 30mer and ran on a 4.8% PAGE gel.

The length of dsDNA required for optimal Rad54 binding is currently is unknown(A). To start addressing this question, we tested double stranded oligos ranging in length from 10 bp to 83 bp. Experiments were carried out with human Rad54, as zebrafish Rad54 did not enter the gel in the electrophoretic mobility assays (EMSAs). Full length Rad54, and the human construct hsΔ1-94Rad54, starting at the position equivalent to residues 88 in zebrafish, were used to study the nucleic acid length dependence. Both proteins began binding DNA fragments at 25 bp, with a maximum affinity being reached around 35bp. Increasing the DNA length further to 40bp did not lead to tighter binding. The 83 bp oligonucleotide showed tightest binding, but additional bands, indicative of multiple Rad54 molecules bound per molecule of DNA were observed.

We further tested if additional binding sites exist in the unstructured region of Rad54. Human full length Rad54, hsΔ1-94Rad54 and hsΔ1-68Rad54 were titrated against a double stranded 31mer(B). Multiple species were observed in the EMSA that were tentatively assigned as higher order complexes with multiple Rad54 molecules bound per DNA molecule, binding of multiple species appeared to be non cooperative. The affinity for full length Rad54, quantified on the basis of the disappearance of the free probe, was on the order of k_D = 1.1 uM +/- 0.350 uM, slightly weaker than that of the hsΔ1-91Rad54 k_D = 0.6 +/- 0.080 uM, which in turn was slightly weaker than the hsΔ1-68 Rad54 construct with a k_D = 0.2 uM +/- 0.06nM. All these affinities were similar, with a slight trend towards tighter binding by the shorter constructs. The unstructured N-terminus does therefore not significantly contribute to DNA binding, and even exhibits a small inhibitory effect. Or results indicate that the minimal binding site required is around 25 bp, with optimal binding strength being reached around 35 base pairs (A).Based on our structural model for DNA binding, the minimal dsDNA length required to bind is around 23bp.