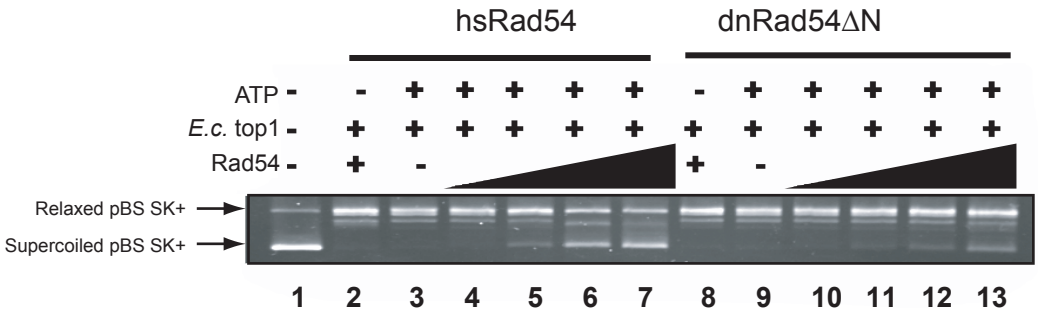
Supplementary Figure 1 The zebrafish Rad54 crystallization construct is active in supercoiling assays.



Zebrafish Rad54 (dnRad54ΔN) introduces supercoiling in closed circular DNA. Incubation of dnRad54ΔN and human full length Rad54 (hsRad54), in the presence of plasmid pSB SK+, *E. coli* topoisomerase I and ATP resulted in the conversion of relaxed to supercoiled DNA, as seen in agarose gel electrophoresis. Protein concentrations used were 0.089, 0.178, 0.35, and 0.71μM for dnRad54ΔN and hsRad54. Each reaction was carried out for 30 min at 30°C in 20 μl of the buffer, containing 25 mM Tris-HCl (pH 7.5), 2.5 mM ATP, 3 mM $MgCl_2$, 1 mM DTT, 2.5 mM phosphoenolpyruvate, pyruvate kinase (20 U/ml), 10% glycerol, 14.2 μM pBluescript SK(+), 1.5 U *E.coli* Topo I. The reactions were stopped by the addition SDS to 0.1%, EDTA to 3mM, and proteinase K to 500 μg/ml. Samples were subsequently analyzed by agarose gel electrophoresis (0.5x TAE, 0.8% agarose).