

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1: Characterization of YacG

The recombinant *E. coli* YacG was overexpressed from pET15b construct in *E. coli* BL26 (DE3) strain. The protein was purified as described previously (Ramelot *et al.*, 2002). The purified fractions were pooled and dialyzed against the buffer containing 50mM Tris-HCl pH-8.0, 0.2% 2-mercaptoethanol, 1mM EDTA, 5% glycerol and stored at -70°C. (A) Purification profile of YacG, Lane 1: Mol. wt. marker, lane 2: Flow through from Ni-NTA column, lane 3 and 4: purified YacG fractions, lane 5: induced cell extract loaded on Ni-NTA column (B) Mass of the recombinant his-tagged YacG determined by MALDI-MS analysis.

Supplementary Figure S2: Gel filtration analysis probing interaction between YacG and DNA gyrase

(A) Elution profiles of protein mixtures loaded on to the Superdex 75 gel filtration column. Protein mixtures (8µg GyrA/GyrB and 16µg YacG) were incubated as described in Experimental Procedures and then loaded. Runs 1-5: GyrB alone, GyrB pre-incubated with YacG, YacG alone, GyrA alone and GyrA pre-incubated with YacG respectively. (B) and (C): SDS-PAGE analyses of Runs 1-3 and 3-5 respectively. Western blots were carried out with anti-His tag, anti-GyrB and anti-GyrA antibodies for detection of YacG, GyrB and GyrA respectively. Panel (i): probing for YacG in its characteristic peak elution volume (12.5ml), Panel (ii): probing for YacG in the void volume (8.5 ml) fractions, Panel (iii): detection of GyrB (Runs 1-3) or GyrA (Runs 3-5) in the void volume (8.5 ml) fractions.