



1	2	3	4	Μ	E1	E2	E3	E4
-	. 1			55 kDa				**
-			• •	40 kDa	-			
1				35 kDa				

## Fig. S1

## Fig. S1. Co-purification of 6His-Scy with ParA and ParA<sub>Scy</sub>. from *S. coelicolor* and the control experiments.

A. The control negative experiment for ParA-Scy co-purification using metal-ion affinity chromatography performed with the extract of thiostrepton-induced strain BD11 (M145pCJW93) strain showing no unspecific interaction of ParA and Scy with the Ni-NA resin. Top panel SDS-PAGE, bottom panel Western-blotting with anti-ParA antibody.

- B. Co-purification of ParA<sub>Scy-</sub> with 6His-Scy from the extract of thiostrepton-induced strain BD10 (ParA<sub>Scy-</sub>, pCJW93p<sub>tipA</sub>*his-scy*) using metal-ion affinity chromatography. Top panel SDS-PAGE, bottom panel Western-blotting with anti-ParA antibody.
- C. Comparison of the efficiency of co-purification of ParA and ParA<sub>Scy-</sub> with 6His-Scy. Westernblotting with anti-ParA antibody of the selected elution fractions. The same amount of the protein was loaded from elution fraction of M145pK48 and from BD10. 1, purified ParA protein; 2, cell extract of M145pK48 (M145pCJW93p<sub>tipA</sub>*his-scy*) strain; 3, cell extract of BD10 (ParA<sub>Scy-</sub>, pCJW93p<sub>tipA</sub>*his-scy*) strain 4, cell extract of J3306 (*ΔparA* strain); M, marker; E1, imidazole elution fraction of M145pK48 (M145pCJW93p<sub>tipA</sub>*his-scy*) – positive result; E2, imidazole elution fractions of BD11 (M145pCJW93) – negative control; E3, imidazole elution fractions of BD10 (ParA<sub>Scy-</sub>, pCJW93p<sub>tipA</sub>*his-scy*) – weakened interaction; E4 imidazole elution fractions of BD12 (ParA<sub>Scy-</sub>, pCJW93) – negative control.



Fig. S2

## Fig. S2. Interaction of ParA mutants with Scy in the bacterial two hybrid system.

Interaction of T18 fused to wild type ParA and mutant: ParAE250V (library), ParA<sub>Scy-</sub> (S249Y, E250V), ParAK44E with wild type ParA and ScyCIV fused with T25. Blue colony color indicates interaction of the proteins analyzed.



Fig. S3. Polymerization of the wild type ParA and ParA <sub>scy-</sub> assayed by glutaraldehyde crosslinking. SDS-PAGE analysis of 5  $\mu$ M ParA crosslinked with the increasing concentration of glutaraldehyde (2.5, 5 and 10 mM) in presence of 2 mM ATP



Fig. S4

Fig. S4. The electrostatic potential on the surface of ParA (A) and ParA<sub>scy</sub>. (B) mapped using VMD. Potential values in kJ/mol.









Fig. S5. Time lapse of *Streptomyces venezuelae* sporulating hyphae development showing close correlation of ParA-EGFP extension and the cessation of the hyphae growth.