**Supplementary Data**

**Table S1**

*Streptomyces coelicolor* strains used in the study

| Strain   | Relevant genotype                                                                 | Sources or reference                  |
|----------|-----------------------------------------------------------------------------------|---------------------------------------|
| M145     | SCP1- SCP2-                                                                       | Bentley et al., 2002                  |
| PS04     | M145 ΔtopA::scar attBΦC31::pIJ6902topA                                            | Szafran et al., 2013                  |
| ASMK01   | M145 ΔhupA::hyg                                                                    | This study                            |
| ASMK03   | PS04 ΔhupA::hyg                                                                    | This study                            |
| ASMK011  | M145 ΔhupA::scar                                                                   | This study                            |
| ASMK031  | PS04 ΔhupA::scar                                                                   | This study                            |
| AK101    | M145 parB-egfp dnaN-mCherry::apra                                                | Kois-Ostrowska et al., 2016           |
| AS11     | AK101 ΔtopA::hyg attB φC31::pIJ6902topA                                            | Strzałka et al., 2017                 |
| AS11.1   | AK101 ΔtopA::scar attB φC31::pIJ6902topA                                            | This study                            |
| ASMK02   | ΔhupA::hyg                                                                         | This study                            |
| ASMK05   | ΔhupA::hyg                                                                         | This study                            |
| ASMK012  | M145 ΔhupA::scar pIJ170hupA-FLAG                                                   | This study                            |
| ASMK032  | PS04 pIJ170hupA-FLAG                                                                | This study                            |
| ASMK034  | PS04 ΔhupA::scar pIJ170hupA-FLAG                                                   | This study                            |
| ASMK015  | M145 ΔhupA::scar pIJ170hupAPAmCherry                                              | This study                            |
| ASMK035  | PS04 ΔhupA::scar pIJ170hupAPAmCherry                                              | This study                            |
| ASMK013  | M145 ΔhupA::scar pIJ170hupA                                                        | This study                            |
| ASMK033  | PS04 ΔhupA::scar pIJ170hupA                                                        | This study                            |
| ASMK016  | M145 ΔhupA::scar pWHM3Hyg                                                          | This study                            |
| ASMK036  | PS04 ΔhupA::scar pWHM3Hgy                                                         | This study                            |
| K306     | M145 hupA-egfp ΔtopA::scar                                                          | Salerno et al., 2009                  |
| AS40     | attBΦC31::pIJ6902topA                                                              | This study                            |
| MS10     | M145 pWHM3Hyg                                                                      | Szafran et al., 2016                  |
| MS11     | PS04 pWHM3Hgy                                                                      | Szafran et al., 2016                  |
| AS41     | M145 pSS170ermhupA                                                                  | This study                            |
| J3337    | M145 dnaN-EGFP                                                                     | Ruban-Ośmiałowska, 2006               |
| AS07     | PS04 dnaN-EGFP                                                                     | This study                            |
Table S2

Oligonucleotides used in the study

| Primer name         | Sequence                                                                 |
|---------------------|--------------------------------------------------------------------------|
| hupA_FW             | GAACGATCCGCAGTCCGCCGACTCCAAGCGGAGCGCTACGTAAT                              |
|                     | TCCGGGGATCCCTCGACC                                                       |
| hupA_RV             | GCCACGCCTCAACGGCAAGAAAGAAACACGGGAGTAACAAACTACGTAT                        |
|                     | GTAGGCTGGAGCTGCTTC                                                       |
| SLIC-hupA-FLAG_FW2  | GACAAAAACTTTAGCATGGAAGTCACACATCATCCACCAG                                 |
|                     | TCCGGGGATCCGTCGACC                                                       |
| SLIC-hupA-FLAG_RV2  | AACCCTAGGGGATCCATCACTTTGCTATCGTTCACATCCTTTGTAATCGATGTC                  |
|                     | ATGAT                                                                    |
| hupA_pam_long_FW    | GAGGTTGAAAAACGCTCACTTGGTACGAACATACATCATCCACCAG                           |
| hupA_pam_long_RV    | TTGCTCAACATGGTAAATTAGTGACACTTGCCCTTGCCGGCTTC                             |
| pam_FW              | TCACTGGAACCTTTAATACATGTTAGCGAAAGGCGAG                                   |
| pam_RV              | CATCGAGCTTGGCCTTTGCGGCTTTCCAAG                                          |
| SCO_HupA_promoter_Fw| AGCTCACTGGGATACCGCAACTACATCATCCACCAG                                   |
| SCO-HupA-FLAG_Rv    | AGCTGGATCCCTTTGCCCCCTTGGCGGCC                                           |
| HupA Sco_fw         | GGATCCATGAACCGCAGTGAGCTGGTGCC                                           |
|                     | TCCGGGGATCCCTCGACC                                                       |
| HupA Sco_rv         | CTGAGCTTGCCTTTGCGGCTTTCCAAG                                           |
| hupA_pss_FW         | TCACTGGAACCTTTAATACATGTTAGCGAAAGGCGAG                                   |
| hupA_pss_RV         | CATCGATTCGCCGACTTAAAGCCTACTTGCCCTTGCGGCC                               |
| RT_sco2950_FW       | CGCGACATCGTCTCCAA                                                       |
| RT_sco2950_RV       | TGCGCTGAAAGTGCAAGGA                                                    |
| hrdBRT_fs           | TGCTCTCCTGAGCTCATTCC                                                   |
| hrdBRT_r            | GTAGGCGCTTGGTGAGTCCAG                                                  |
| ermhupA_FW          | AGGAGGCCCCCATATGGAAGTATCACTGAAACCGCAGTGAGCTGG                           |
| ermhupA_RV          | GACTCTAGTTAATTAATCCTACTTGTCCCTTGCGGCC                                |
Strains construction and protein purification

Construction of hupA deletion strains

To construct the S. coelicolor strain lacking the hupA gene, first we constructed a E59 cosmid derivative containing hygromycin resistance cassette instead of hupA gene using primers hupA_FW and hupA_RV and PCR targeting method, which yielded E59 ΔhupA::hyg cosmid. Secondly hygromycin resistance cassette was removed by SnaBI digestion and religation to create E59 ΔhupA::scar cosmid. In this cosmid ampicillin resistance cassette in SuperCos was replaced with hygromycin resistance cassette containing oriT site, necessary for conjugation into S. coelicolor.

In order to complement hupA deletion a 769 bp long fragment containing hupA gene and its promoter sequence was amplified using hupA_FW and hupA_RV primers and then cloned into pIJ170 at KpnI site with the SLIC method yielding pIJ170 hupA plasmid. After verification with sequencing this vector was introduced into ASMK011 (ΔhupA::scar) and ASMK031 (ΔhupA::scar, TopA depletion) strains in order to obtain ASMK013 and ASMK033 strains, respectively.

In order to remove hygromycin resistance cassette from AS11 (TopA*, parb-egfp, dnaN-mcherry) strain we used a H5 ΔtopA::scar cosmid. After conjugation colonies sensitive to hygromycin and kanamycin were obtained indicating a successful double crossing-over yielding strain AS11.1.

The cosmid E59ΔhupA::hyg was introduced into S. coelicolor strains M145, PS04 (TopA-controlled), AK101 (parb-egfp, dnaN-mcherry) and AS11.1 (TopA-controlled, parb-egfp, dnaN-mcherry), hygromycin resistant and kanamycin sensitive clones were selected indicating a successful double crossing-over. The obtained strains: ASMK01 (ΔhupA::hyg), ASMK03 (ΔhupA::hyg, TopA depletion), ASMK02 (ΔhupA::hyg, parb-egfp, dnaN-mcherry) and ASMK05 (ΔhupA::hyg, TopA-controlled, parb-egfp, dnaN-mcherry) were verified by PCR. In order to remove hygromycin resistance cassette from ASMK01 and ASMK03 strains we used a E59 ΔhupA::scar cosmid. After conjugation colonies sensitive to hygromycin and kanamycin were obtained indicating a successful double crossing over yielding strains ASMK011 (ΔhupA::scar) and ASMK031 (ΔhupA::scar, TopA*), which were verified using PCR.

HupA-FLAG construction

773 bp long fragment containing hupA gene with its promoter sequence was amplified with primers SCO_HupA_promoter_Fw and SCO-HupA-FLAG_Rv. The obtained sequence was cloned into the pGEM-SMC-FLAG plasmid at NcoI-BamHI sites using the SLIC method, replacing the SMC gene. Then fragment containing hupA-FLAG gene and promoter sequence was amplified using SLIC-hupA-FLAG_FW2 and SLIC-hupA-FLAG_RV2 primers and cloned into pIJ170 integrative plasmid at XmaJI site using the SLIC method yielding pIJ170 hupA-FLAG plasmid. This vector, after verification with sequencing, was used to transform strain ASMK011 (ΔhupA), PS04 (TopA*) and ASMK031 (ΔhupA TopA*). Obtained colonies resistant for hygromycin were verified using Western blot for production of HupA-FLAG protein yielding the strains ASMK012, ASMK032 and ASMK034.

HupA-PAmCherry construction

First, 751 bp long fragment containing PAmCherry gene was amplified with pam_FW and pam_RV primers and cloned into pIJ170-FLAG at XhoI and XmaJI site to obtain pIJ170 PAmCherry vector. Next, a 769 bp long fragment containing hupA gene and its promoter sequence was amplified using hupA_pam_long_FW and hupA_pam_long_RV primers and then cloned into pIJ170 PAmCherry at KpnI site with the SLIC method yielding pIJ170 hupAPamCherry plasmid. After verification with sequencing this vector was introduced into ASMK011 (ΔhupA::scar) and ASMK031 (ΔhupA::scar, TopA*) strains in order to obtain ASMK015 and ASMK035 strains respectively.

ermhupA construction

First, 322 bp long fragment containing hupA gene was amplified with ermhupA_FW and ermhupA_RV primers and cloned into pIJ10257 (contains constitutive promoter erm) vector digested with XhoI using the
SLIC method. After verification with sequencing pIJ10257 ermhupA vector was introduced into M145 strain yielding strain AS41.

TopA* dnaN-EGFP and TopA* HupA-EGFP strains construction

First, pIJ6902 ptiptopA plasmid was introduced to strains K306 (Salerno et al., 2009) and J3337 (Ruban-Ośmiałowska, 2006). Than topA gene was deleted using H5topA::scar vector yielding strains AS40 (ptiptopA ΔtopA::scar hupA-EGFP) and AS07 (ptiptopA ΔtopA::scar dnaN-EGFP).

TopA_{sv} purification
E. coli strain containing pET28topAsv was used for protein production. For protein overproduction, cells were grown to OD600 ~ 0.4 at 37°C, then isopropyl-β-d-thiogalactopyranoside (IPTG) was added to a final concentration of 0.3 mM and the culture was continued for 4 h at 37°C. The cells were collected by centrifugation, re-suspended in 50 mM NaH_{2}PO_{4}, pH 8.1, 300 mM NaCl with 20 mM imidazole and sonicated. Fast protein liquid chromatography (FPLC) system with HisTrap HP columns (GE Healthcare) was used to purify recombinant proteins from cell lysate, followed by desalting using Zeba Spin Desalting Column (Thermo Scientific) equilibrated with 50 mM NaH_{2}PO_{4}, pH 8.1, 300 mM NaCl, 10% glycerol buffer. Protein samples were stored in -80°C.
A. Purification of HupA-GST protein. M – marker, 1 – culture lysate, 2 – cell pellet, 3-4 – column wash, 5-8 wash after PreScission protease treatment, 9 – HupA protein after desalting.

B. TopA relaxation assay followed by incubation with HupA. Two hundred nanograms of supercoiled plasmid pUC19 was initially incubated with TopA (30-120 nM) for 15 minutes, followed by the addition of HupA (0–8 μM) and incubation for 15 min at 20°C. Topoisomers were resolved without deproteinization by agarose gel electrophoresis.

C. TopA relaxation assay in the presence of HupA. Two hundred nanograms of supercoiled plasmid pUC19 was initially incubated with TopA (60-90 nM) and HupA (0–4 μM) for 15 minutes. Topoisomers were resolved without deproteinization by agarose gel electrophoresis.

The positions of the supercoiled and relaxed topoisomers are indicated.
Fig. S2

A. Growth curves of the control strain (M145, purple), ΔhupA hupA-FLAG strain (ASMK012, green) and ΔhupA hupA-PAmCherry (ASMK015, yellow), cultured in ‘79’ medium.

B. Growth curves of the TopA-depleted strain (PS04, purple) and TopA-depleted ΔhupA hupA-FLAG (TopA ΔhupA hupA-FLAG, ASMK034, yellow), cultured in ‘79’ medium with various concentrations of topA inducer (thiostrepton: 0 or 0.2 μg/ml).

C. Growth curves of the TopA-depleted strain (TopA*, PS04, purple), TopA-depleted hupA-FLAG strain (TopA* hupA-FLAG, ASMK032, green) and TopA-depleted ΔhupA hupA-PAmCherry strain (TopA* ΔhupA hupA-PAmCherry, ASMK035, yellow), cultured in ‘79’ medium in the presence of a topA inducer (thiostrepton 0.2 μg/ml).

D. Growth curves of the ΔhupA (ASMK011, yellow) and control (M145, purple) strains, cultured in ‘79’ medium in the presence of gyrase inhibitor (novobiocin: 0 or 10 μg/ml).

E. Growth curves of the control strain (M145, purple), ΔhupA strain (ASMK011, blue), ΔhupA hupA-FLAG (ASMK012, green) and ermhupA strain (AS41, yellow), cultured in ‘79’ medium.

The semitransparent lines show the mean absorbance values obtained from five replicates, and the bold lines correspond to the fit of the loess model.
A. HupA-EGFP fluorescence measured in native SDS PAGE gel using Azure 600 (Biosystems). Cell lysates from the TopA-depleted hupA-EGFP strain (TopA*, AS40) were prepared after 18 h of culture at 30°C. The protein concentration in each lane was normalized.

B. HupA-EGFP fluorescence measured in native SDS PAGE gel using Azure 600 (Biosystems). Cell lysates from the TopA-depleted hupA-EGFP strain (TopA*, AS40) compared to control strain hupA-EGFP (K306) were prepared after 48 h of culture at 30°C. The protein concentration in each lane was normalized.

C. Western blot results produced with an anti-FLAG antibody using cell lysates of strains ΔhupA (ASMK011), ΔhupA hupA-FLAG (ASMK012), TopA-depleted hupA-FLAG (TopA* hupA-FLAG, ASMK032) and TopA-depleted ΔhupA hupA-FLAG (TopA* ΔhupA hupA-FLAG, ASMK034) cultured for 48 h in ‘79’ medium.

D. RT-PCR results of hupA gene expression in strains: wild type (M145), ΔhupA (ASMK011), ΔhupA hupA (ASMK013), ΔhupA hupA-FLAG (ASMK012), ΔhupA hupA-PAmCherry (ASMK015) and ermhupA (AS41). Strains were cultured in YEME/TSB medium for 24h. Each sample was performed in triplicate. Statistical analysis was performed using ANOVA with Tukey post-hoc test, statistical significance is given against the wild type strain.

E. Topoisomers distribution of plasmid pWHM3Hyg isolated from 48 h of culture of the control strain (MS10), TopA-depleted strain (TopA*, MS11), ΔhupA strain (ASMK016) and TopA-depleted ΔhupA strain (TopA* ΔhupA, ASMK036). The thiostrepton concentration (range 0-1 μg/ml) is indicated below the image.
Fig S4

Comparison of AT% percent calculated for ChIP-seq HupA binding sites identified by edgeR (left) and MACS3 (right) and the same number of random *S. coelicolor* sequences of similar length, p-values calculated by two-sided Wilcoxon test is shown on the plot.
A. Time-lapse DIC snapshots of germinating spores of the control strain (AK101, left panel) and TopA-depleted ΔhupA strain background (TopA* ΔhupA, ASMK05, right panel). The yellow arrow indicates a lysed germinating spore, while the red arrows indicate hyphae with arrested growth. Scale bar 5 μm.

Fig. S5

A. Time-lapse DIC snapshots of germinating spores of the control strain (AK101, left panel) and TopA-depleted ΔhupA strain background (TopA* ΔhupA, ASMK05, right panel). The yellow arrow indicates a lysed germinating spore, while the red arrows indicate hyphae with arrested growth. Scale bar 5 μm.
B. Time-lapse DIC snapshots of 24 h hyphae of the control strain (AK101, top panel) and TopA-depleted strain (TopA*, AS11, bottom panels) overlaid with green fluorescence channels showing the ParB-EGFP complexes. Scale bar 1 μm.

C. Images of vegetative hyphae (DIC, grey) and DNA stained with DAPI (blue) of the Δ*hupA* (ASMK011), TopA-depleted (TopA*, PS04), TopA-depleted Δ*hupA* (TopA* Δ*hupA*, ASMK031, thiostrepton 0.01 μg/ml) and wild-type (M145) strains. Red arrows indicate hyphae lacking DNA in the tip proximal region. Scale bar 2 μm.

D. 7 days colony surface images of control strain (M145) and TopA-depleted Δ*hupA* strain (TopA* Δ*hupA*, ASMK031). Scale bars 10 μm.

E. Boxplots showing comparison of the distance between hyphal tip and the edge of DAPI signal in strains: control strain (M145, 65 hyphae), Δ*hupA* (ASMK011, 41 hyphae), TopA-depleted (TopA*, PS04, 66 hyphae) and TopA-depleted Δ*hupA* (TopA* Δ*hupA*, ASMK031, thiostrepton 0.01 μg/ml, 80 hyphae)
Fig. S6

Variability of the number of ParB complexes in germinating *S. coelicolor* hyphae in the control strain (AK101), TopA-depleted strain (TopA*, AS11), Δ*hupA* strain (ASMK02) and TopA-depleted Δ*hupA* strain (TopA* Δ*hupA*, ASMK05) (all in *parB-egfp* and *dnaN-mcherry* genetic background). The number of visible ParB complexes was compared between each consecutive time point (10 minutes), and the percentage of detectable ParB complex loss or gain was calculated.
A. Snapshots from the time-lapse analysis of the ParB-EGFP (green) and DnaN-mCherry complexes (red) in vegetative hyphae of the control strain (AK101) and TopA-depleted strain (TopA*, AS11). The fluorescence images are next to the DIC images (grey). Scale bar 1 μm.

B. Positions of the ParB-EGFP complexes adjacent to the tip (tip-proximal ParB1 – green and tip-distal ParB2 – blue) and DnaN-mCherry complexes (red) in hyphae (grey bar) of the control strain (AK101, 30 hyphae) and TopA-depleted strain (TopA*, AS11, 20 hyphae). For each timepoint, a mean with a 95% confidence interval is plotted.

C. Average distance between the ParB complexes after duplication over time in the control strain (AK101, black) and TopA-depleted strain (TopA*, AS11, red). Lines show the linear model with 95% confidence intervals.

D. Percentage of hyphae in which duplicated ParB complexes could be detected at the indicated time after replisome appearance in the control strain (AK101, black) and TopA-depleted strain (TopA*, AS11, red). Error bars show 95% confidence intervals.

Fig. S7
A. Snapshots from the time-lapse analysis of the DnaN-EGFP (green) in germinating spores of the control strain (J3337) and TopA-depleted strain (TopA*, AS07). The fluorescence images are overlayed with the DIC images (grey). Scale bar 1 μm.

B. Number of DnaN-EGFP complexes divided by hyphae length over time for the control strain (J3337, black, 30 hyphae) and TopA-depleted strain (TopA*, AS07, red, 30 hyphae). Shown curve was fitted using loess algorithm.

C. Average fluorescence intensity of DnaN-EGFP complexes over time for the control strain (J3337, black, 30 hyphae) and TopA-depleted strain (TopA*, AS07, red, 30 hyphae). Shown curve was fitted using loess algorithm.