

Figure S1. *S. coelicolor* growth in presence of novobiocin. The wild type *S. coelicolor* (M145) was cultured in the presence of increasing concentrations of novobiocin (0 - 20 µg/mL) in liquid 79 medium at 30°C. The culture growth was monitored using the Bioscreen C instrument. The optical density of the culture was measured at 600 nm wavelength in 20-minute intervals. Each conditions were analyzed in triplicate and subsequently averaged values with standard deviations were plotted against time of culture.

Growth curves

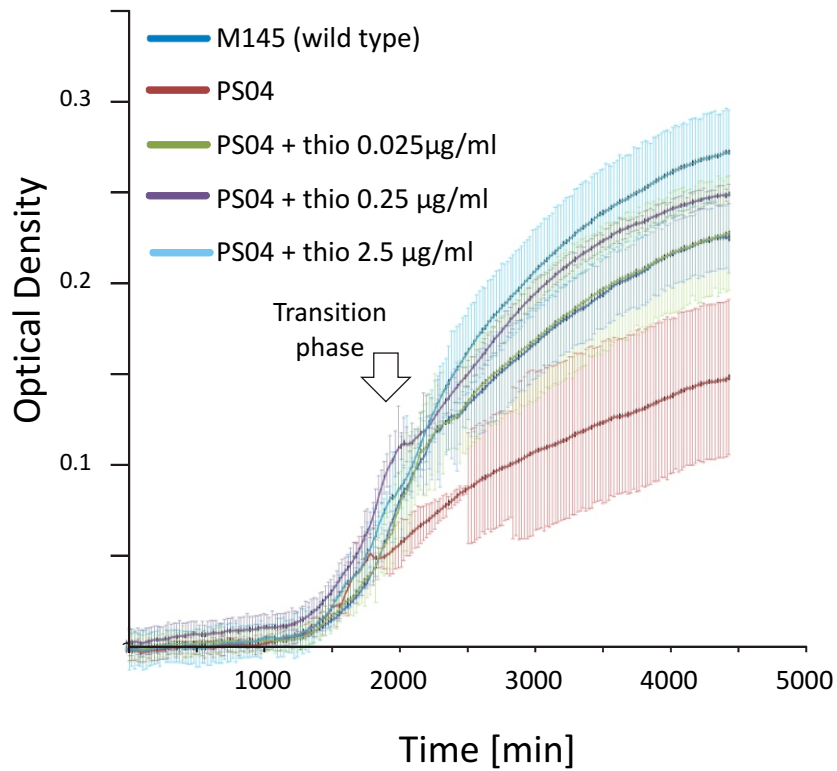


Figure S2. *S. coelicolor* strain PS04 growth in presence of thiostrepton. The PS04 strain was cultured in the presence of increasing concentrations of thiostrepton (0 - 2.5 µg/mL) in liquid YEME/TSB medium at 30°C. The culture growth was monitored using the Bioscreen C instrument. The optical density of the culture was measured at 600 nm wavelength in 20-minute intervals. Each conditions were analyzed in triplicate and subsequently averaged values were plotted against time of culture. The wild type M145 strain served as the control in the experiment. The transition phase during *S. coelicolor* vegetative growth was marked with arrow. Each conditions were analyzed in triplicate and subsequently averaged values with standard deviations were plotted against time of culture.

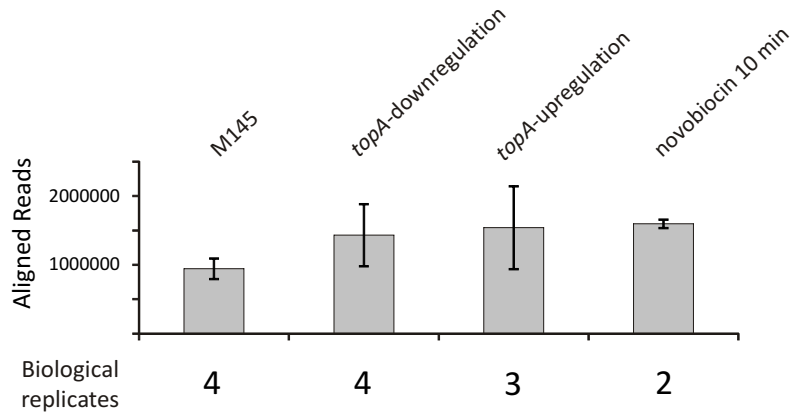


Figure S3. The number of aligned reads in the RNA-Seq experiments. The reads obtained from MiSeq Illumina sequencing of the libraries for the wild type strain as well as the strains with modified DNA supercoiling conditions (*topA* gene upregulation and downregulation, as well as the wild type strain exposed for 10 minutes to novobiocin 10 $\mu\text{g/ml}$) were aligned using Rockhopper software. Below the diagram, the number of replicates for each tested condition is shown.

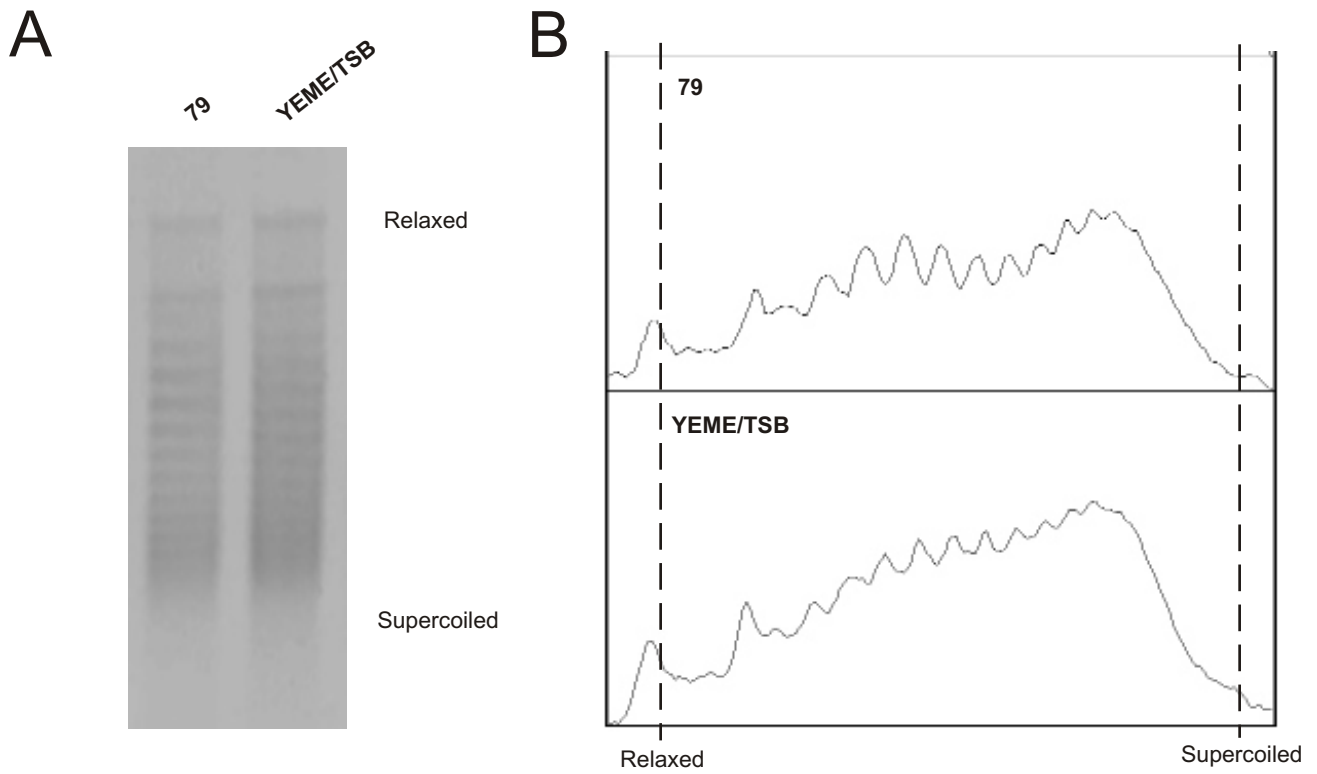


Figure S4. The reporter plasmid supercoiling under different growing conditions. (A) Plasmid supercoiling assay, in which the reporter plasmid was isolated from the *S. coelicolor* MS10 strain growing in liquid 79 and YEME/TSB medium, respectively. **(B)** The analysis of topoisomer distribution of the reporter plasmid using Fiji software.

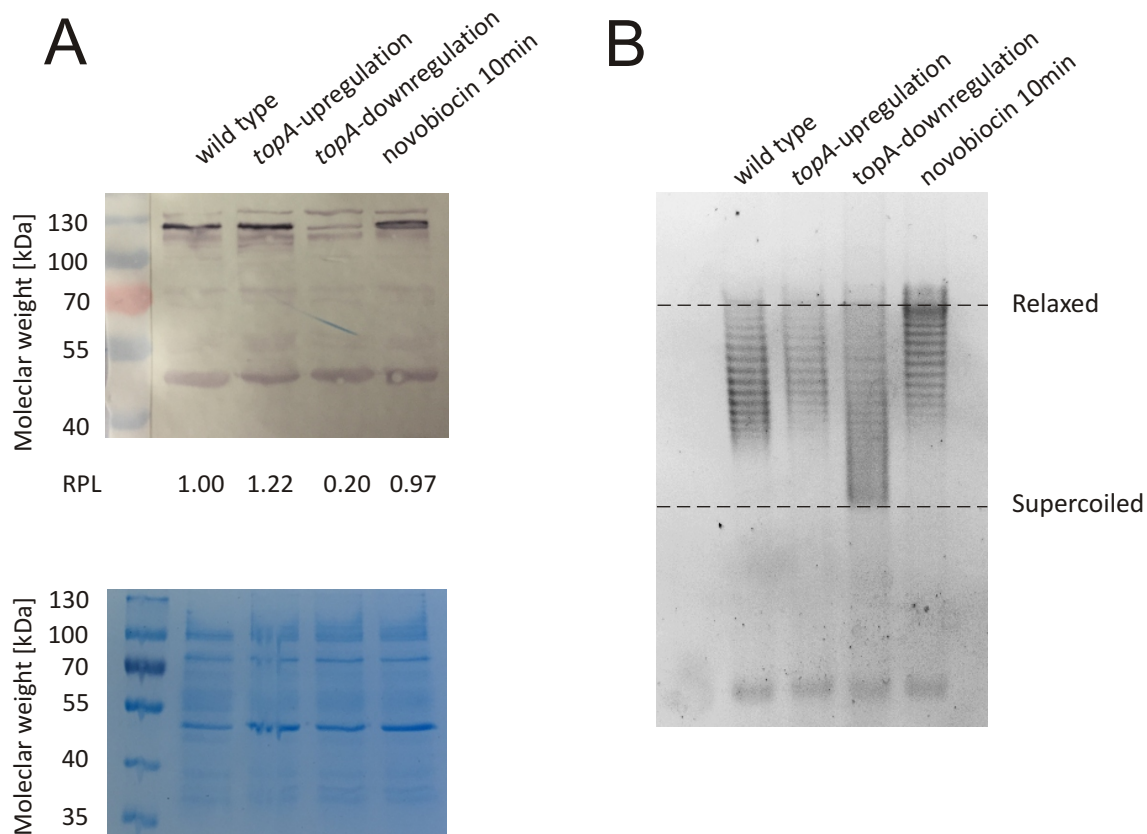


Figure S5. TopA levels and DNA supercoiling under different experimental conditions. (A) Western blot analysis of *S. coelicolor* TopA levels using anti-TopA polyclonal antibodies (upper panel). The loading control for the experiment (lower panel). The relative protein level (RPL) was calculated as the TopA band intensity in comparison to the wild type TopA level estimated as 1.00 (B) Plasmid supercoiling assay, in which the reporter plasmid was isolated from the control strain (MS10) and the strain with modified *topA*-transcription (MS11) (upregulation and downregulation of the *topA* gene) or the control strain (MS10) treated with novobiocin.

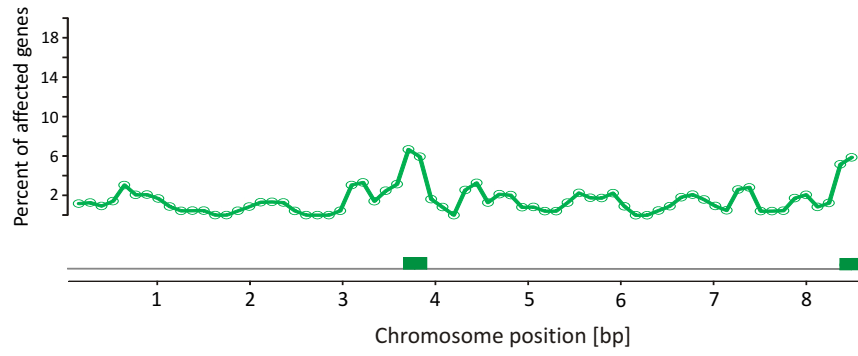
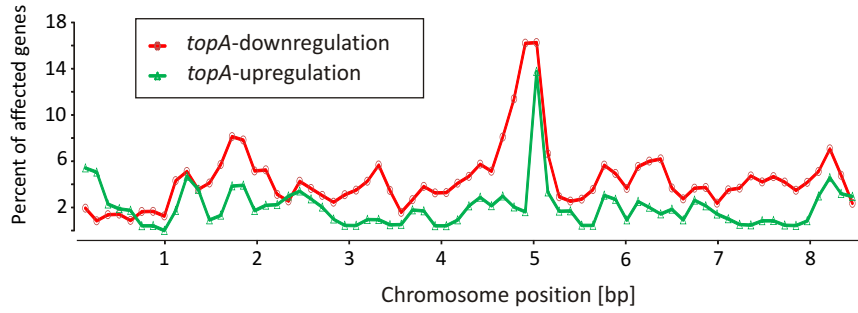
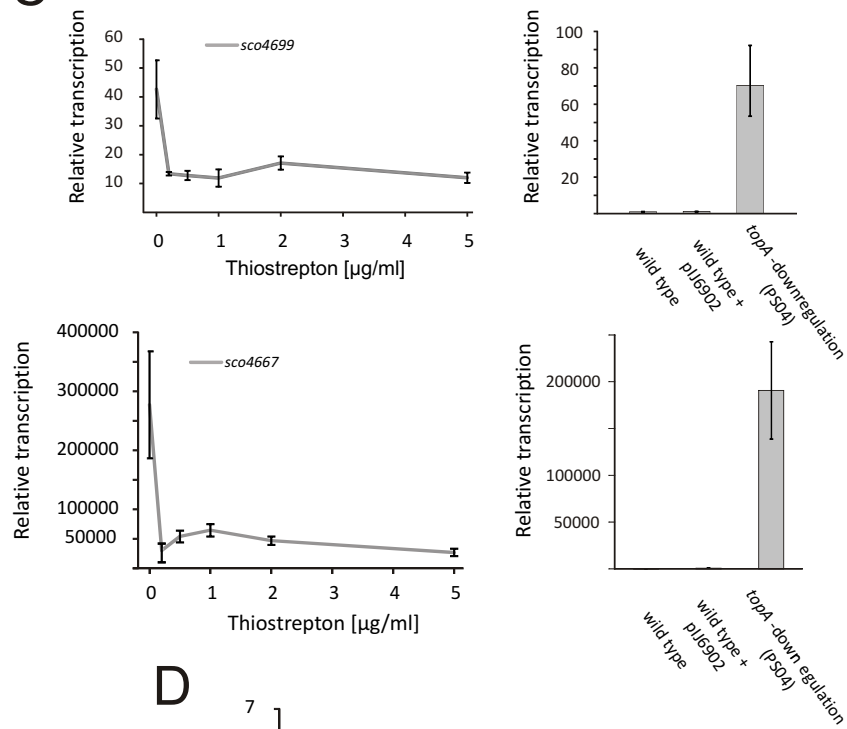
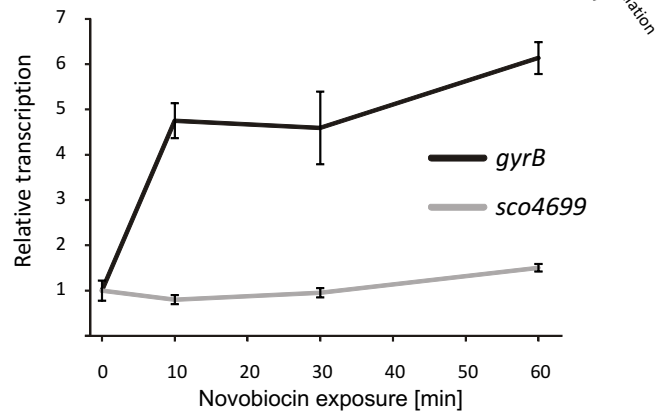
A**B****C****D**

Figure S6. Supercoiling-sensitive clusters. (A) Identification of novobiocin-sensitive clusters. The percent of genes upregulated in response to novobiocin was calculated in 250-kbp windows (with a sliding window of 125 kbp) and subsequently the mid of the window was plotted versus chromosome position. The regions containing more than 5% of the upregulated genes are indicated in green and are deemed to be novobiocin-sensitive clusters. (B) Overlay of the plots showing the percentage of upregulated genes in the 250-kbp genome fragment identified in the *topA*-downregulated and *topA*-upregulated strains. (C) RT-qPCR analysis of *sco4699* (upper panel) and *sco4667* (lower panel) transcription at different concentrations of *topA* gene inducer. The wild type level of any given transcript is normalized to 1. Plots on the right show the relative transcription levels of *sco4699* and *sco4677* genes in the TopA-depleted and the wild type strains as well the control strain containing the integrative pIJ6902 vector. (D) Relative transcription of the *gyrB* gene and *sco4699* gene after exposure (0-60 minutes) to novobiocin at a final concentration of 10 $\mu\text{g/ml}$. The transcript enrichment for *gyrB* and *sco4699* was normalized by the expression of the *hrdB* gene which served as the endogenous control and compared to the wild type strain in which transcription of analyzed genes was estimated as 1.

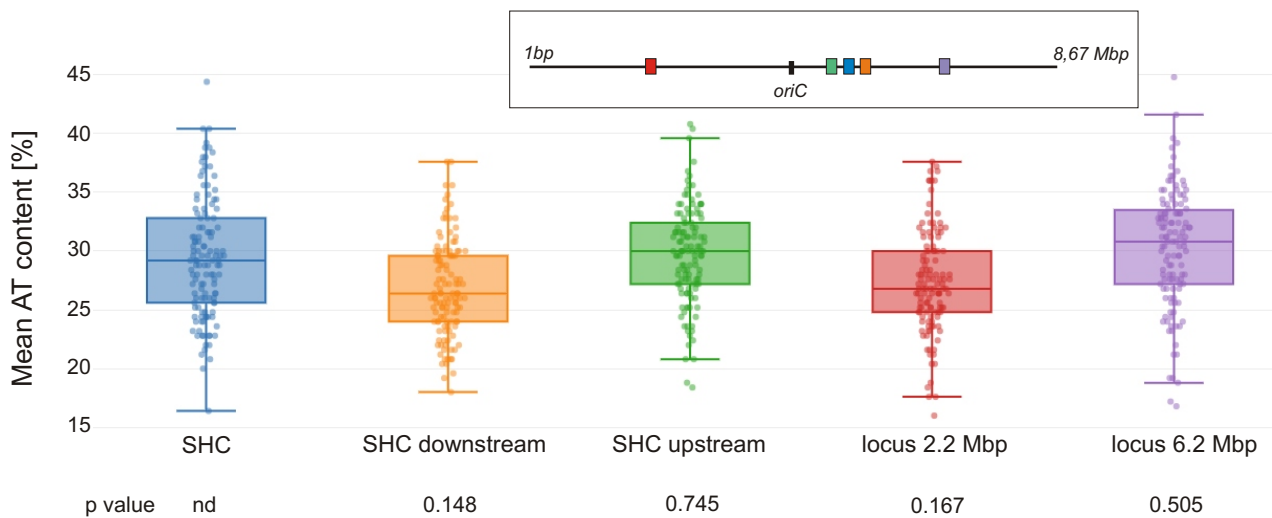


Figure S7. The box plot analysis of AT content within 30 kbp chromosomal regions. The AT percentage was calculated within each of 250 bp windows covering 30 kbp region of *S. coelicolor* chromosome including: SHC region (blue), SHC downstream region (yellow), SHC upstream region (green), both region separated from SHC region by 5 kbp spacer region, as well as two distinct chromosomal fragments in positions 2.2 Mbp (red) and 6.2 Mbp (violet) of the linear chromosome. The scheme presenting the chromosomal position of all tested regions is shown above. The difference in mean AT content was calculated using Mann-Whitney test. The calculated p value for each test is shown below the box plot.

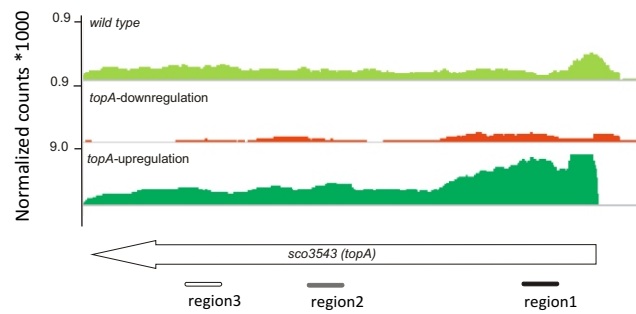


Figure S8. The *topA* gene transcription and TopA binding. RNA-Seq data visualization using Integrated Genome Viewer showing the number of successfully aligned fragments within the *topA* gene in the *S. coelicolor* wild type strain (light green), the TopA-depletion strain (orange) and the *topA*-induced strain (dark green). Note the Y-axis scale difference. The positions of the 1-3 regions tested in RT-qPCR (See also Fig. 4D) are marked

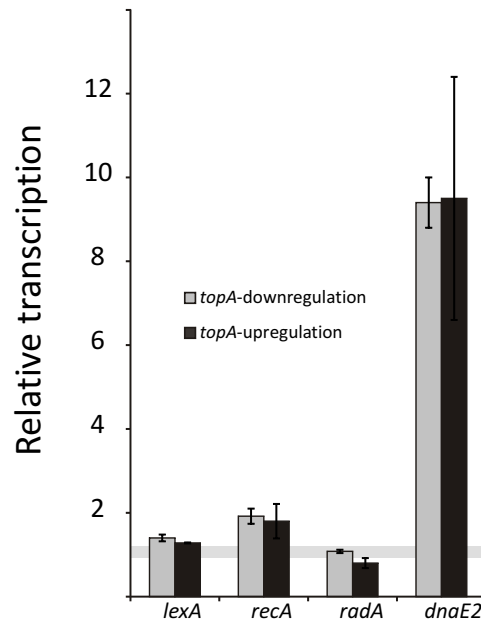


Figure S9. RT-qPCR analysis of the relative transcript level of genes belonging to the putative LexA regulon in *S. coelicolor* (*lexA*, *recA* and *dnaE*) under TopA-depletion (gray) and *topA*-induction (black) conditions. The *radA* gene, which does not belong to the *lexA* regulon, served as the negative control. The transcript enrichment for particular genes was normalized by the expression of the *hrdB* gene which served as the endogenous control and compared to the wild type strain in which transcription of analyzed genes was estimated as 1.