Supplementary Material

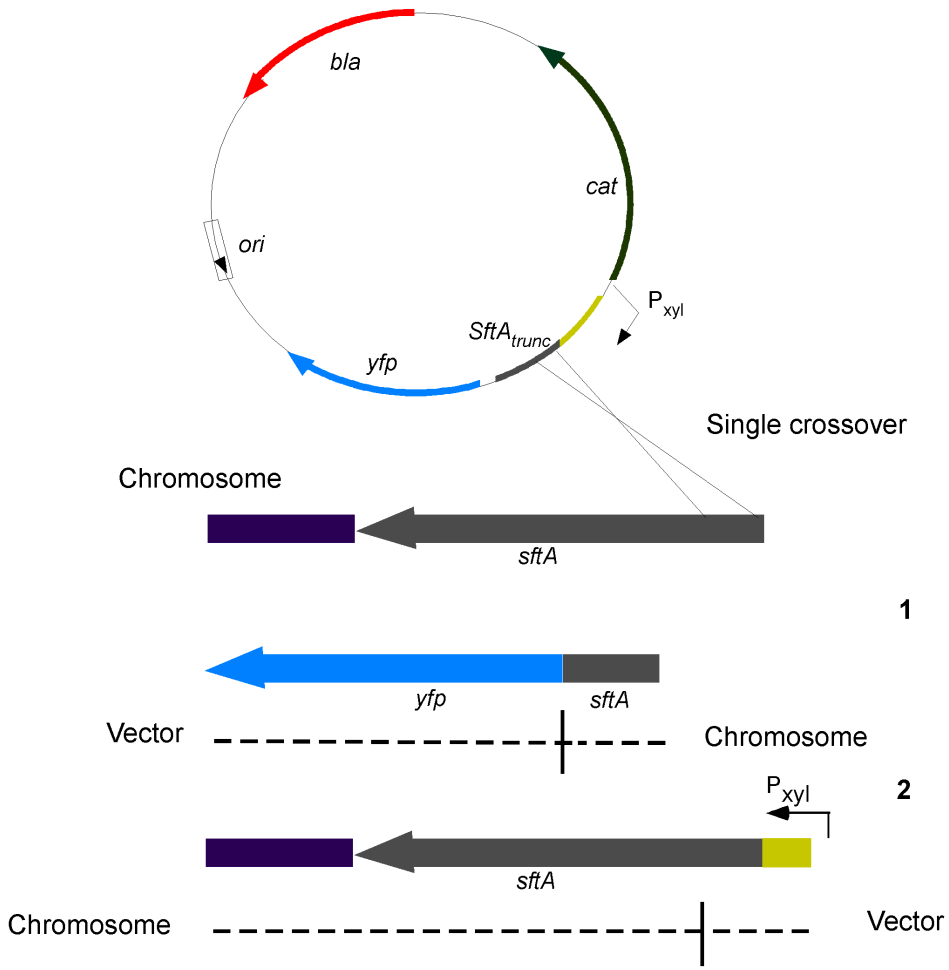

Figure S1. Map for the plasmid integration of pSG1164 [Kidane et al., 2004] truncation containing plasmid. A 500 base pair fragment was cloned between the *Apa*I an *Eco*RI cutting sites. The truncation is shown in the map as a dark grey line. In cases where the truncation was sorter than 500 bp, the rest of the fragment length was cloned from the sequence upstream of the ORF (yellow line). The single crossover resulted in a truncated copy (Part **1**), and in the full length protein being translated downstream of the xylose promoter (Part **2**).

Table S1. List of oligonucleotides used in this study

Oligonucleotide	Sequence	Construct
2342	5’-CATGGGCCCGACGAACCGAAATCCGCG-3’	1164-sftA$_{(1-246)}$-yfp
2343	5’-CATGAATTCTTGCTCTTCGGCTTGTTCA-3’	
2473	5’-CATGGGCCCTAGCGGCTTGGTTTCTGC-3’	1164-sftA$_{(1-137)}$-yfp
2474	5’-CATGAATTCAACAGATGGTTTTTGATTG AAT-3’	
2410	5’-CATGGGCCCTTAGAAGGAGACTATGAGG-3’	1164-sftA$_{(1-105)}$-yfp
2411	5’-CATGAATTCTGTATGCTGCTC TTCTTCA-3	
2468	5’-CATGGGCCCAAGGATGCAGAGCTTCGTG-3’	1164-sftA$_{(1-67)}$-yfp
2469	5’-CATGAATTCGTATCCGTCAGGCA-3’	
2471	5’-CATGGGCCCGAAACGCTTCAGATCGT	1164-sftA$_{(1-34)}$-yfp
2472	CTG-3’ 5’-CATGAATTCTACTTCTTGTTGCT-3’	
2527	5’-CATGGGCCCAAGTTGTCGTTGCC-3’	1164-sftA$_{(1-50)}$-yfp
2528	5’-CATGAATTCATATATTTTAGGAT-3’	
2523	5’-CATGGGCCCATGAGTTGGCTTCATAAA	1193-amyE::sftA$_{(1-439)}$-yfp
2526	TTTT-3’ 5’-CATGAATTCCGGAAATACATAGCTGCC-3’	
2579	5’-CATGGGCCCCCGGGAGACAAAA CCCGCT-3’	1193-amyE::sftA$_{(21-439)}$-yfp
2619	5’-CATGGGCCCAGTCGTGCCTGA-3’	1193-amyE::sftA$_{(61-439)}$-yfp
1250	5’- GCGCTTTCTCATAGCTCACGCTGTAGGTATCT CAGTTCGGTGTAGGTCGTTCGCTCCAAG-3’	60 bp fragment -KOPS
1251	5’- CTTGGAGCGAACGACCTACACCGAACTGAGA TACCTACAGCGTGAGCTATGA-3’	
1882	5’- TATATTGGGTAGGGAATTATAGGGCAGGGAAT ATTGGGAAGGGTATATGGGGAGGGGAA TA-3’	60 bp fragment +KOPS
1883	5’- TATTCCCTCCCCATATACCCTTCCCAATATTCC CTGC CCTATAATTCCCTACCCAATATA-3’	

Supplementary Materials and Methods

Overexpression and purification of SftA

For purification of SftA, 1 l of autoinduction medium [Studier, 2005] was inoculated with 10 ml overnight culture of *E. coli* BL21 carrying the corresponding expression vector. The 1l culture was then incubated for 12 h at 25°C. Autoinduction is based on the diauxic behavior of *E. coli*. The preferred sugar, in this case glucose, is consumed first, leading to rapid growth, followed by a lag phase. In the lag phase, the cellular machinery used to metabolize the second sugar, lactose, is activated which concomitantly induces overexpression. Cells were then harvested by centrifugation for 10 min at 5000 rpm (Sorvall RC-6+ centrifuge, F94 rotor) and washed two times with Tris buffer (0.1 M Tris-HCl, pH 8.0, 0.1 M NaCl) and stored at -80°C. Prior to use, cell pellets were thawed and resuspended in 10-40 ml of Tris buffer containing a mix of protease inhibitors (complete, Roche). Cells were lysed by two passages through a French press at approximately 20000 psi (Aminco), and lysates were cleared by centrifugation for 30 min at 14000 rpm at 4°C (SS-34 rotor) for two times.

Affinity chromatography was performed using an ÄKTA Prime chromatography equipment (GE Healthcare) and a Ni-chelating column (HisTrap, 1 ml column volume). Before injection, cell lysates were passed through a filter (Filtropur S, pore size 0.45 µm) to prevent clotting of the column. The sample was loaded in several steps and, after extensive washing to remove contaminants, specifically bound protein was eluted in a gradient (20 ml at 1 ml min^{-1}) of 0-0.5 M imidazol in Tris buffer. Fractions containing SftA were pooled and subjected to gel filtration.

Gel filtration was performed using an ÄKTA FPLC (GE Healthare) and a Superose 6 (10/300 GL) column. Gel filtration columns were equilibrated in Tris buffer, and fractions from affinity chromatography were injected in 100 µl to 2 ml steps and eluted at a constant flow rate of 0.5 ml min^{-1}. Fractions containing SftA were pooled and stored at 4°C until they were used for further experiments. The final concentration of SftA varied from 0.5 to 1.2 g l^{-1}, corresponding to a yield 1 to 2.5 mg per liter of culture.

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References

Studier FW: Protein production by auto-induction in high-density shaking cultures. Protein expression and purification 2005;41:207-234.

Kidane D, Sanchez H, Alonso JC, Graumann PL: Visualization of DNA double-strand break repair in live bacteria reveals dynamic recruitment of *bacillus subtilis* recf, reco and recn proteins to distinct sites on the nucleoids. Mol Microbiol 2004;52:1627-1639.