Supplemental Materials

Table S1. Strains and plasmids.

Strain or plasmid	Description	Reference
Strains		
M145	Wild type *S. coelicolor* A3(2)	(1)
GSA3	M145 Δ*ssgA*	(2)
GSB1	M145 Δ*ssgB*	(3)
Plasmids		
pHJL401	*Streptomyces*/*E. coli* shuttle vector (5-10 and around 100 copies per genome, respectively)	(4)
pGWS271	pHJL401 harboring *ssgB*(Stro) and its upstream region	This study
pGWS294	pHJL401 harboring *ssgB*(Sery) and its upstream region	This study
pGWS295	pHJL401 harboring SACE5535 and its upstream region	This study
pGWS299	pHJL401 harboring *ssgB*(Krad) fused to the *S. coelicolor ssgB* promoter	This study
pGWS554	pHJL401 harboring *ssgB*(Tfus) fused to the *S. coelicolor ssgB* promoter	This study
pGWS555	pHJL401 harboring *ssgB*(Fran) fused to the *S. coelicolor ssgB* promoter	This study
pGWS556	pHJL401 harboring Francci3_3418 fused to the *S. coelicolor ssgB* promoter	This study

Table S2. Oligonucleotides

Primer name	**DNA sequence (5’ to 3’)***	**Location 5’ end[+]**
Sery_BF	CTG*GAATTC*GCGAGCGGGAATTCGATCT	-399
Sery_BR	CTG*AAGCTT*CGGCTGGGCTCTTGATCTC	+563
Sery_2F	CTG*GAATTC*GCAACCTTCTGAACGCCGTG	-408
Sery_2R	CTG*AAGCTT*CGATTCCAGTGCCCGGGAAG	+496
SalTr_BF	CTG*GAATTC*TCCACTGAGGATCGCTCCGTCAC	-296
SalTr_BR	CTG*AAGCTT*CATCACCACCCACGGCTGAGC	+569

* Underlined sequences indicate non-homologues sequence added to create restriction sites (in italics) at the ends of the PCR fragments.

[+] Relative to the first nucleotide of the respective gene

Table S3. *ssgB* genes used in the genetic locus comparison (see Figure 1) .

Genome tag or contig number	**Organism**	**GeneID**	**Name used in this work**
SCO1541	*Streptomyces coelicolor*	1096967	
SAV6810	*Streptomyces avermitilis*	1217088	
scab0975f02.p1k	*Streptomyces scabies*	-	
SGR5997	*Streptomyces griseus*	-	
FRAAL2127	*Frankia alni* ACN14a	4235729	
Francci3_1359	*Frankia* sp. CcI3	3904606	*ssgB*(Fran)
Francci3_3418*****	*Frankia* sp. CcI3	3905658	Francci3_3418
Franean1_5158	*Frankia* sp. EAN1pec	5675752	
SACE_1961	*Saccharopolyspora erythraea*	4941816	*ssgB*(Sery)
SACE_5535*****	*Saccharopolyspora erythraea*	4945278	SACE5535
Acel_1369	*Acidothermus cellulolyticus*	4486262	
Krad_3069	*Kineococcus radiotolerans*	5336467	*ssgB*(Krad)
Noca_2368	*Nocardioides*	4595986	
Tfu_2111	*Thermobifida fusca*	3581046	*ssgB*(Tfus)
Strop_1600	*Salinispora tropica*	5058058	*ssgB*(Stro)
Sare_1560	*Salinispora arenicola*	5704920	

***** The locus of this gene does not resemble that of *ssgB* gene orthologues (not shown in **Fig. 1**)

Table S4. Viable spore counts of transformants. For explanation of viable counts, see Figure 3 and Experimental Procedures. Percentages of viable spores are given, with the total number of spores counted per transformant between brackets.

Strain	Plasmid	Plasmid-encoded SALP	Viable spore count #
M145	pHJL401	none	100% (319)
GSB1	pHJL401	none	no spores
GSB1	pGWS271	*ssgB*(Stro)	65% (152)
GSB1	pGWS294	*ssgB*(Sery)	80% (311)
GSB1	pGWS295	SACE5535	no spores
GSB1	pGWS299	*ssgB*(Krad)	87% (150)
GSB1	pGWS554	*ssgB*(Tfus)	93% (224)
GSB1	pGWS555	*ssgB*(Fran)	99% (149)
GSB1	pGWS556	Francci3_3418	96% (70)

Fig. S1

Nucleotide sequences of *ssgB* from *S. coelicolor* and of the actinomycete *ssg* genes described in the paper. *Sac. erythraea ssgB* (SACE_1961) and SACE_5535 and *Sal. tropica ssgB* (Strop_1600) were produced by PCR amplification from genomic DNA, using primers (Table S2 in the paper) designed against the underlined sequences. *Frankia* sp. cci3 *ssgB* (Francci3_1359) and Francci3_3418, *K. radiotolerans ssgB* (Krad_3069), and *T. fusca ssgB* (Tfu_2111) were produced by gene synthesis, whereby the upstream region of *S. coelicolor ssgB* (italicized) was fused to the coding sequences to ensure correct developmental transcription (in terms of timing and expression level) of the actinomycete ORFs. Highlighted triplets: green, most likely translational start site; red, translational stop codon; cyan, originally predicted translational start of *S. coelicolor ssgB*, but less likely due to downstream located promoter (Kormanec and Sevchikova, 2002). All genes were cloned into the low-copy number vector pHJL401 making use of the engineered *Eco*RI (GAATTC; upstream) and *Hin*dIII (AAGCTT; downstream) restriction sites, except SACE_1961 (*Eco*RI-*Bam*HI (GGATCC)). Considering the uncertainty regarding the start site of *ssgB* from *Kineococcus radiotolerans* [SsgB(Krad)], the annotated (long) ORF was used.

>ssgB(Scoe)
[SCO1541; nt positions 1650212-1650830]
GAATTCCTGTCACCGGAATGGGCGTACGACTCGAACGGTCCATCGGCCGGAAGAGTTACG
TAAACACACGCAAAACACTCAGAGGGGTTTACAACGGCACCGTAGGTGGCATGTCGATTT
*CGCCGACGTGCGAATCCCCGAGCGCACACTGAGCGAAAGGCCCTGGCGCTT*ATGAACACC
ACGGTCAGCTGCGAGCTGCACCTGCGCCTCGTTGTGTCGAGCGAGTCCTCCCTGCCTGTC
CCCGCAGGCCTGCGGTACGACACGGCCGACCCCTACGCCGTGCACGCCACCTTCCACACC
GGAGCCGAGGAGACCGTCGAGTGGGTCTTCGCCCGCGACCTGCTCGCCGAAGGTCTCCAC
CGTCCCACCGGCACCGGCGACGTCCGTGTCTGGCCGTCCCGCAGTCACGGCCAGGGCGTC
GTGTGCATCGCTCTCAGCTCCCCGGAGGGCGAGGCACTGCTCGAGGCCCCGGCGCGGGCC
CTGGAGTCCTTCCTGAAGCGCACAGACGCCGCCGTGCCCCCCGGCACGGAACACCGGCAC
TTCGATCTCGATCAGGAGCTCTCGCACATCCTGGCGGAAAGCTAGGGCGGGGCCTCGCGT
GAAGCCGCCCGTCGCCGTCGACTCGAAGCTT

Predicted gene product (137 aa):
MNTTVSCELHLRLVVSSESSLPVPAGLRYDTADPYAVHATFHTGAEETVEWVFARDLLAE
GLHRPTGTGDVRVWPSRSHGQGVVCIALSSPEGEALLEAPARALESFLKRTDAAVPPGTE
HRHFDLDQELSHILAES

>SsgB(Tfus)
[Tfu_2111; gene coordinates 2471763-2472179]
GAATTCCTGTCACCGGAATGGGCGTACGACTCGAACGGTCCATCGGCCGGAAGAGTTACG
TAAACACACGCAAAACACTCAGAGGGGTTTACAACGGCACCGTAGGTGGCATGTCGATTT
*CGCCGACGTGCGAATCCCCGAGCGCACACTGAGCGAAAGGCCCTGGCGCTT*ATGAGCAGC
AGCGGCACCTCTATCACCTGCGAAGTCGGTTTGCAGCTGATCGTTCCTGATCGTGCCCCC
GTGCCCCTGGTGGCCCGTCTCGACTACTCCGTGGACGACCCTTACGCCATCCGGGCGGCC
TTCCATGTGGGCGACGACGAACCCGTGGAGTGGATCTTCGCTCGTGAGCTGCTGACCGTG
GGCATCATCCGCGAAACAGGCGAAGGCGACGTGCGCATCTGGCCCTCGCAGGACGGGAAG
GAGCGCATGGTCAACATTGCCCTCTCCTCACCTTTCGGCCAGGCGCGGTTCCACGCGCAG
GTGGCACCCCTCTCGGAGTTCCTGCACCGCACCTACGAGCTGGTTCCTGCCGGGCAGGAG
AGCGACTACATCGACATCGACGCTGAAATCGCTGAGCACCTCAGCTAGTCTCACCGGGGT
CGGGACCGCTGTCCAGATCTAAGCTT

Predicted gene product (138 aa):

MSSSGTSITCEVGLQLIVPDRAPVPLVARLDYSVDDPYAIRAAFHVGDDEPVEWIFAREL
LTVGIIRETGEGDVRIWPSQDGKERMVNIALSSPFGQARFHAQVAPLSEFLHRTYELVPA
GQESDYIDIDAEIAEHLS

>ssgB(Fran)
[FranCci3_1359; gene coordinates 1632539-1632970 (reverse strand)]
GAATTCCTGTCACCGGAATGGGCGTACGACTCGAACGGTCCATCGGCCGGAAGAGTTACG
TAAACACACGCAAAACACTCAGAGGGGTTTACAACGGCACCGTAGGTGGCATGTCGATTT
*CGCCGACGTGCGAATCCCCGAGCGCACACTGAGCGAAAGGCCCTGGCGCTT*ATGAACACC
ACGGTCAGCTGCGAACTCGCCCTCCGCCTTGTCGTTCCCGGCGGGGCGCCCGTGCCGGTC
GCCGCCACCGTCCGGTACGAGCCGGCCGATCCGTACGCGATCAGCATCGGCTTCCGCACC
GGTGCGGACGAGGTCGTGGAGTGGACATTCGCGCGCCAGCTCCTCAGCGACGGTGTGCGC
CGACCTGCCGGGGATGGCGACGTGCAGGTGTGGCCTGCCGCCCAGTCCGGGGGACGGATC
GTCTGCCTGTCCCTGTCCAGCCCGTCCGGGCATGCACTGTTCGAGATGCCGCGGTCCGAG
GTGCTGGCCTTCCTACGGCGGACGTATTCAGCCGTCCCCCTCGGCGGGGAGAGCGACATC
ATCGATCTTGACGCCGAACTCGCCCTCCTCATCTGGGGCGGCCCCGAGCGGTGATCTCAC
CGGGGTCGGGACCGCTGTCCAGATCAAGCTT

Predicted gene product (143 aa):
MTIRHDSITAELALRLVVPGGAPVPVAATVRYEPADPYAISIGFRTGADEVVEWTFARQL
LSDGVRRPAGDGDVQVWPAAQSGGRIVCLSLSSPSGHALFEMPRSEVLAFLRRTYSAVPL
GGESDIIDLDAELALLIWGGPER

>FranCcI3_3418
[FranCci3_3418; gene coordinates 4062259-4062696 (reverse strand)]
GAATTCCTGTCACCGGAATGGGCGTACGACTCGAACGGTCCATCGGCCGGAAGAGTTACG
TAAACACACGCAAAACACTCAGAGGGGTTTACAACGGCACCGTAGGTGGCATGTCGATTT
*CGCCGACGTGCGAATCCCCGAGCGCACACTGAGCGAAAGGCCCTGGCGCTT*ATGCTCCGT
CACAGCTCGACGCCCGCGCATGTCGTGGCGCGCGATCTATCCGTTGACTACGTCACGGAG
GAGGGGGAGCGAGAGGCGATCCCCGGCACACTCCGTTATGACCCGGCTGATCCCTTCGCC
GTTGAGTTGATCCTACGTCCCGGTGAAGAGGCCATCACCTGGATCTTCGCCCGCGCGCTG
CTGGCCGAAGGGATCGAGGTTCCCGCCGGGACCGGCGACGTGCGCATCCGGCCGACGAGA
TCCAAGGGGCAGACGGTGATCACGGTGTCCCTGTCCAGCCCGTCGGGGCATGCCGATCTC
GAGCTGGCGAAGTCGAAGGTCACCGCCTTTCTCGCCGACTGCCACGGACAGGTTCCAGCG
GGGCGGGAGAGCAACGGCATCGACTGGAACAACGAACTTCACATGCTGCTGCGCACTCGG
AACACCTGATCTCACCGGGGTCGGGACCGCTGTCCAGATCTAAGCTT

Predicted gene product (145 aa):
MLRHSSTPAHVVARDLSVDYVTEEGEREAIPGTLRYDPADPFAVELILRPGEEAITWIFA
RALLAEGIEVPAGTGDVRIRPTRSKGQTVITVSLSSPSGHADLELAKSKVTAFLADCHGQ
VPAGRESNGIDWNNELHMLLRTRNT

>ssgB(KRad)
[Krad_3069; gene coordinates 3147086-3147583]
GAATTCCTGTCACCGGAATGGGCGTACGACTCGAACGGTCCATCGGCCGGAAGAGTTACG
TAAACACACGCAAAACACTCAGAGGGGTTTACAACGGCACCGTAGGTGGCATGTCGATTT
*CGCCGACGTGCGAATCCCCGAGCGCACACTGAGCGAAAGGCCCTGGCGCTT*ATGGGAAAC
TCATGGACACGGTCGCTGGAGCCCGTCAGCGGCCATGAGCACACGGAGGTCGACGTGCCT
GCACTGCCGCACCGCAAGACCGAGGTCGACGTCGACGTGTCCTTGCGGCTCCGGACCACC
AGCGGTCCGGGACTCCCCGTCCCCGCCAGCCTCCACTACGGAGCTGACGACCCGTACGCC
ATCCACGCGGTCTTCCGCGGCGGCGACACGGACGTGGAGTGGGTCTTCGCCCGGGACCTG
TTGAGGGAGGGGCTGTCCGCACCCGCCGGCGCCGGCGACGTGCAGGTGCAGCCCTGCTCC
GACTCCCCCGACGGCCGTCCCCGGGTCCTGCTGCGCCTGTCGTCCCCGGACGGCAACGCG

GAGCTCGAAGCGGACGAGTCCGACGTGCGGCGCTTCCTGCGTCGCGCCGACGCCCTCGTC
CCCCCCGGCCGCGAGACCCGCCACCTGGACCTCGACGAGCTCATCGCCCGTCTCGTCTCC
TGATCTCACCGGGGTCGGGACCGCTGTCCAGATCTAAGCTT

Predicted gene product (163 aa):
VGNSWTRSLEPVSGHEHTEVDVPALPHRKTEVDVDVSLRLRTTSGPGLPVPASLHYGA
DDPYAIHAVFRGGDTDVEWVFARDLLREGLSAPAGAGDVQVQPCSDSPDGRPRVLLRLSS
PDGNAELEADESDVRRFLRRADALVPPGRETRHLDLDELIARLVS

>SsgB(Sery)
[SACE_1961; gene coordinates 2141781- 2142206]
GAATTCGATCTCGCCGGGCGGCGTTGTCCGACGAACGCGATGGGTTCGACCCGGGCGCGA
CCGCGCAGGCGGTGGCGTGTGGGAACTGCCAGTGGCCGATCGCCGGGTGGCGACGGGGCG
CGGGCTCCGGTCGGCGTCCGCCGGAGGTGCGGACGGCCCATTCGGCGCTAGGGGATCGGC
AGGGCTTGCCGGGGGCGGCGGACCCGTTGCCGGGTCCGGTCGGAAAAGAGCGGGTGAACA
CGCTGCGCATTGCCTTGGTTGCCCAGCGAATTGGGGCCTGCCCCGTACGGGTGATCTATC
CGCGAGTTGCGAGCCATCGGGCCCAGGAAGGCGTCACAACTGTGTTGCTCGGTCGATAAG
CGGACCGGAAGGTAGTAGGAAGGCGAGGACAATGCGTAACGATCACGTGACACTCCGTTC
AACGGCGGTGTTCGATCTGCTGGCCCCGCAGACGCCGGCCGTGCCGGTGCAGGTCGAGCT
GCGCTACGACACTCGTGACCCCTACGCCGTGGTGGCCGCGTTCCGCACCGGCCGCGCGGG
GTGGGTCGAATGGGTCTTCGCGCGCGATCTGCTGGCCGACGGCCTCATCGCGCACGCGGG
CGTGGGCGATGTGACCATCCGCCCCGCGGTCGACGACCCCGAGGTCGTGGTGATCGAGCT
GAGCTCCCCCTCCGGGCACGCCGTGTTCGAGGCTTCGGCGCAGGAGCTGGCCGACTTCCT
GGACCGCACCTACGACGTGGTGGTCCCCGGCAACGAGAACCTGTGGGTCAACGTCGACGA
CGCGCTCACCCGCCTGTTGCCGCACGACCTGTCCTGACCGGCCCGCACCGGGTCTGGCCA
GGCCGAACGCTGCTCGGAACCGGGTAGCGGGACCCCGGTTCGATCGCCGTTGGCGGCGTG
GTCTAAAGTTTCTCGTGCAACGCGGCGGACGGCCGGAGATCAAGAGCCCAGCCGGGCTCG
GAGATCGAAGTCGGCAAGCCGCAGGCAGGCGGACGTAGCGCAGCTGGTAGCGCATCACCT
TGCCAAGGTGAGGGTCGCGGGTTCGAGTCCCGTCGTCCGCTCTGGATGAGTCAGGTCCAG
CCCGTTTCGGATTGGATCC

Predicted gene product (141 aa):
MRNDHVTLRSTAVFDLLAPQTPAVPVQVELRYDTRDPYAVVAAFRTGRAGWVEWVFARDL
LADGLIAHAGVGDVTIRPAVDDPEVVVIELSSPSGHAVFEASAQELADFLDRTYDVVVPG
NENLWVNVDDALTRLLPHDLS

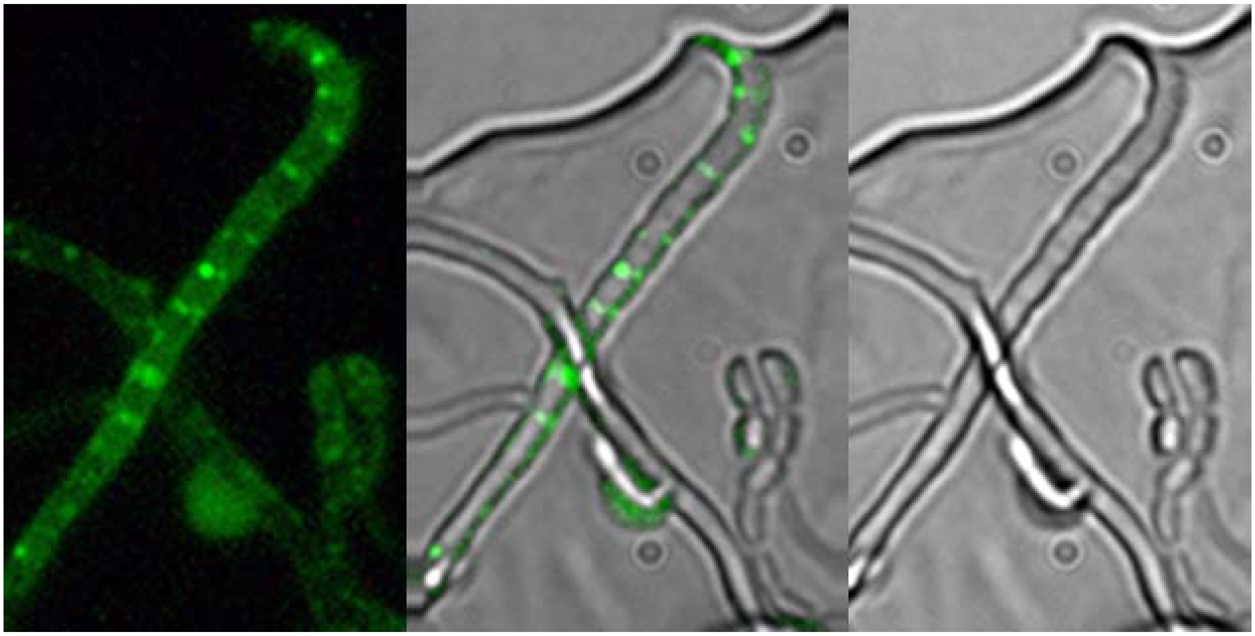
>SACE_5535
[SACE_5535; gene coordinates 6233273- 6233695 (reverse strand)]
GAATTCGCAACCTTCTGAACGCCGTGGCCGGGGATCCGACGGGCGCGGCTGGAGCTCCAT
CGCACTCCGCTCTTTCCAAGGCCACCAAGCGCCTCGTGAGCCAAAATCACGCCTCCGTCG
CCCATTCTGGATATTGACGTTCGTGGCTGGTCGCATAAACTTGTGCCTTACTCGCGGTTT
CCCGTCTGCACGGTGAACCACAGGGGACCAAACTGCCCGGTAGGGTGAAGGCCCTTGGTT
CAGGTGTGCCCTCGGCTCCCGTAACGCCTGTTCGAAAGGGCACTTCGTGTAACGCGTGTC
CGGGTGGGTCGCCCCGTGTACGTCGGCGGACCTGAAGACTTCGTGAAGAAGATATGGATT
CGGGGCGTGCATACTCGGAACTCGATACGGAGTCGAGGGAAGAGGAGCACGCGAATGAAG
AACCACAGGACCGTCCTTACCAGCATGGTGTTCAAGCTGCTGGCGCCGGCGGGAGCGACC
GCGCCGGTCGGCGTGGAGCTGCGGTTCGAGAGCCGCAACCCGTACGAGGTGTCGCTGGCC
TTCAGCGCTGGTAGCGGCGGTCACGTGGAGTGGGTGCTCGCCAGGGACCTGCTGGCCGAC
GGTCTGATGATCGACAGCGGGGAAGGTGACGTGCGGGTGGGGCCCGACGCGAGCGATCCC
GCGGTGATCGTGGTGTCGCTGTCCTCGCCGAGCGGGCAGGCCACCTTCGAAGCCGACGCT
GAGCAGTTGGTCGACTTTTTGAACCGGACCTACGACGTGGTACCGCCTGGTAAGGAACAC
CAGTGGATGAGTATCGACGAGGCCCTGGCGCGGATGCTGCCGCATGACCTGTACTGATCG
CCGCGGGTCCGGCGCCGGATCGCCGGATGTGCCTCGCCGCTAGTCCGGGGCTTCCCGGGC
ACTGGAATCGAAGCTT

Predicted gene product (140 aa):
MKNHRTVLTSMVFKLLAPAGATAPVGVELRFESRNPYEVSLAFSAGSGGHVEWVLARDLL
ADGLMIDSGEGDVRVGPDASDPAVIVVSLSSPSGQATFEADAEQLVDFLNRTYDVVPPGK
EHQWMSIDEALARMLPHDLY

>SsgB(Stro)
[Strop_1600; gene coordinates 1821027..1821458 (reverse strand)]
TCCACTGAGGATCGCTCCGTCACTTTTCCTCCCCCAGTGTGGCGCGCGCCCGTCACTGCG
ACCGTGGACTGGACGTTACCGGAGTAGTCGACGACTGAAGCACCTGATGCCGATCGGCAC
CGCGGACGGGCAGAACCGGAAATCCTACAGGAACTTTCACATTCTGGAGGACACTCCGAA
GACCTTCCCACCACTGGGTGAAGTCAGCGTATGGCGGAGCGTAGCTAGGGGCAGCACCTG
AGTATGGGAAAAGCGGGACACGCGCGTCCCGCAGCGGTGCCGGGGGGAGAACGTCCATGA
GTGTCATCCGACCGACGACCGTAGAGGTCGAGACGTCGCTAAGGCTCGTCGCACCTGACG
CCACGGCCTTGCCGGTGCGCGCCAGTCTGCGCTACGACCCTGCTGACCCGTATGCCGTCC
ACGTCCTGTTCCATGCGGAATCGGCTGGTGGCGAGGCGGTCAGCTGGTCGTTCGCACGGG
AACTGCTGGTGACCGGCCTCGATGAGCCGGCCGGCATCGGCGATGTGCGGGTCTGGCCCT
GGGCCACCCCGCGCGGGGACTTCGTCGCCCTGGCGTTGTCGTCCCCAGATGGCAACGCCC
TGTTTGAGGTGCCGCGGAGCGTCTTGGTGCGCTTCCTGCGGCGGACCTACGTCGTCGTCG
CGCGCGGCCGGGAGGCCGAGCACCTGGACGTCGACACGGCGGTGAACCGGCTGCTCGCCG
GACGCTGAGATCGAAGGGGCTGGAGTCTGGTGACTGGTCAGCCGGGGCCGCGCGGAGCTG
CCGAGTCCGCGCGACCCCGGCTACCCCGGTCGGTACGCGAAGCGGTGCGCCGCGGATGCG
CCCGGCTCAGCCGTGGGTGGTGATGAAGCTT

Predicted gene product (143 aa):
MSVIRPTTVEVETSLRLVAPDATALPVRASLRYDPADPYAVHVLFHAESAGGEAVSWSFA
RELLVTGLDEPAGIGDVRVWPWATPRGDFVALALSSPDGNALFEVPRSVLVRFLRRTYVV
VARGREAEHLDVDTAVNRLLAGR

Fig. S2. Fluorescence micrographs showing localization of SsgB-GFP to emerging sporulation septa in sporogenic aerial hyphae of *S. coelicolor* FM145. Left: fluorescence micrograph showing ladders of SsgB-GFP; right: light micrograph of the same image; middle: overlay of left and right images. Note that the prominent aerial hypha containing the SsgB-GFP foci shows constrictions precisely at the sites to which SsgB-GFP localizes (see right panel). FM145 is a low fluorescent variant of *S. coelicolor* M145 (5).



Supplemental References

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