SUPPLEMENTAL INFORMATION

Belonging to the manuscript:

Constitutive expression of *ftsZ* overrides the *whi* developmental genes to initiate sporulation of *Streptomyces coelicolor*

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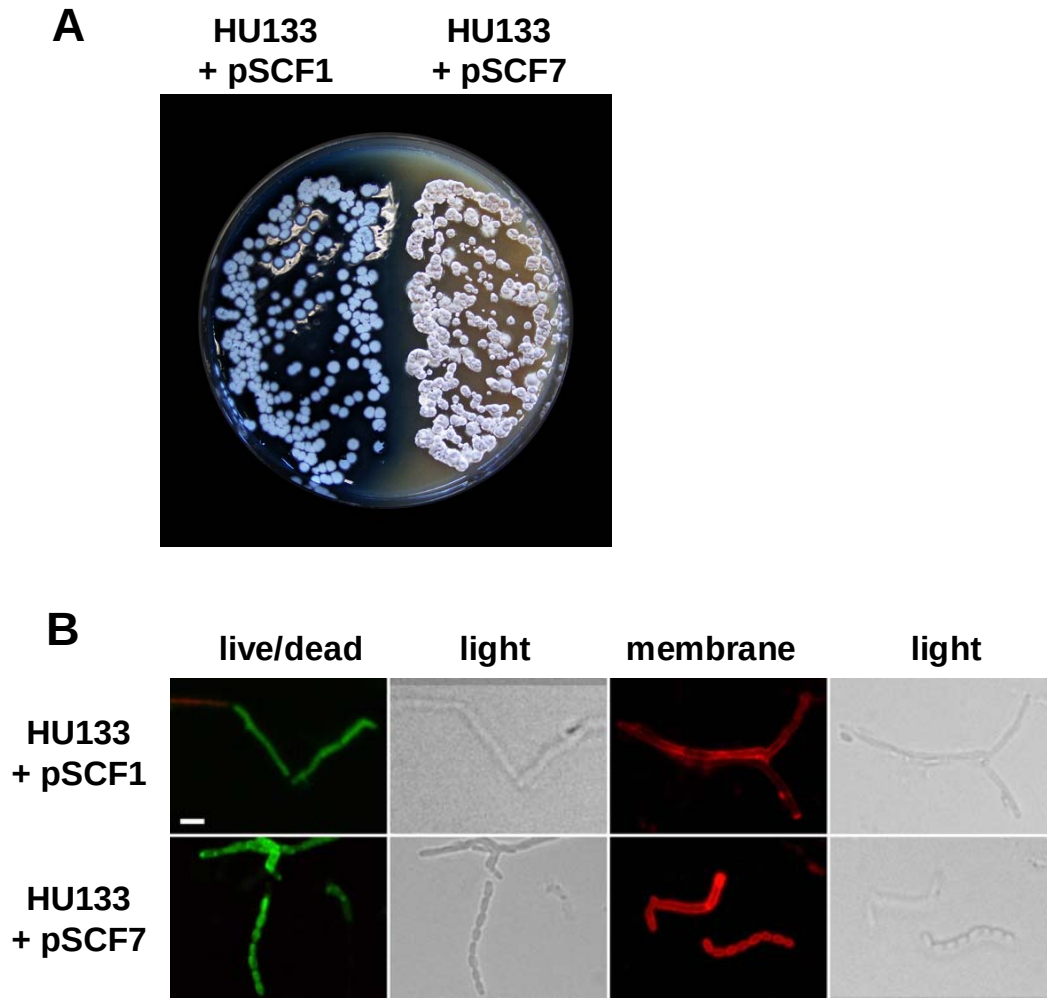

Figure S1. Complementation of the *ftsZ* null mutant HU133 by pSCF7.
(A) Transformants of the *ftsZ* null mutant HU133 harbouring control plasmid (left) or pSCF7 (right), with the latter expressing *ftsZ* from the constitutive *ermE* promoter. Note that grey pigmentation is restored to the *ftsZ* mutant. The *ftsZ* null mutant formed flat colonies and strongly overproduced actinorhodin; normal colony morphology and actinorhodin production were also restored by the introduction of pSCF7. The strains were grown for 6 days on SFM agar plates at 30^0C.
(B) Live/dead staining (left) and septum staining (right) for the *ftsZ* null mutant harbouring control plasmid (top) or pSCF7 expressing FtsZ (bottom). Live cells were identified with syto-82 (green), dead cells with propidium iodide (red). Septa were highlighted by the membrane stain FM5-95. For all images both fluorescence and light images are presented. Bar, 2 μm.

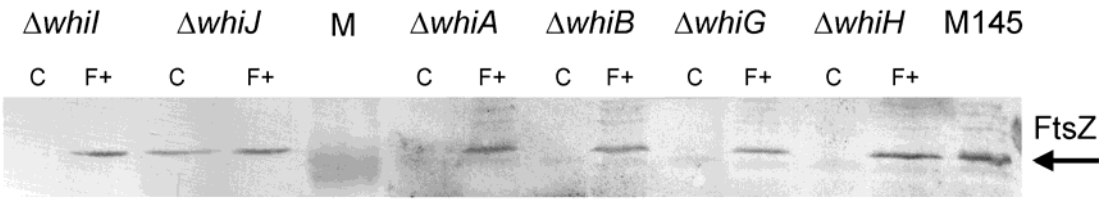

Fig. S2. Western analysis of protein extracts obtained from liquid-grown mycelia. Samples were *whi* mutants harbouring control plasmid (lanes labelled 'C') or plasmid pSCF7 expressing FtsZ (lanes labelled 'F'). A protein extract from *S. coelicolor* M145 is shown as the control.

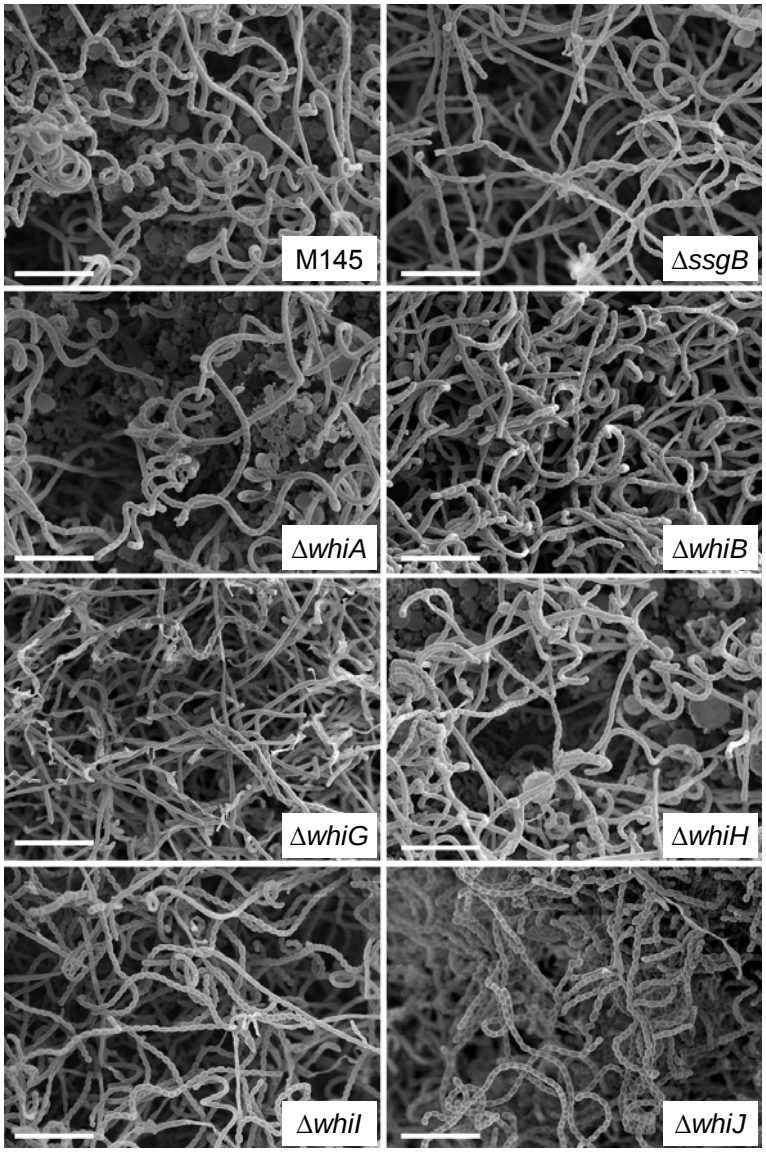

Figure S3. Cryo-scanning electron micrographs showing overviews of sporogenic aerial hyphae of *whi* mutants harbouring pSCF7. Strains were grown for 3 days on SFM agar plates to visualise aerial hyphae and emerging spore chains. As detailed in Fig. 3, expression of FtsZ restored sporulation to all *whi* mutants. Note that while sporulation is restored to *whiG* null mutants, aerial hyphae show lysis, and sporulation occurs with reduced frequency; the *ssgB* mutant produced occasional deformed spore-like bodies as compared, most likely as a result of the incorrect localization of FtsZ in the absence of SsgB. Note that *whiJ* transformants hypersporulated, with very long spore chains and hardly any non-sporulating aerial hyphae. For further details see Fig. 3. Bar, 10 μm.

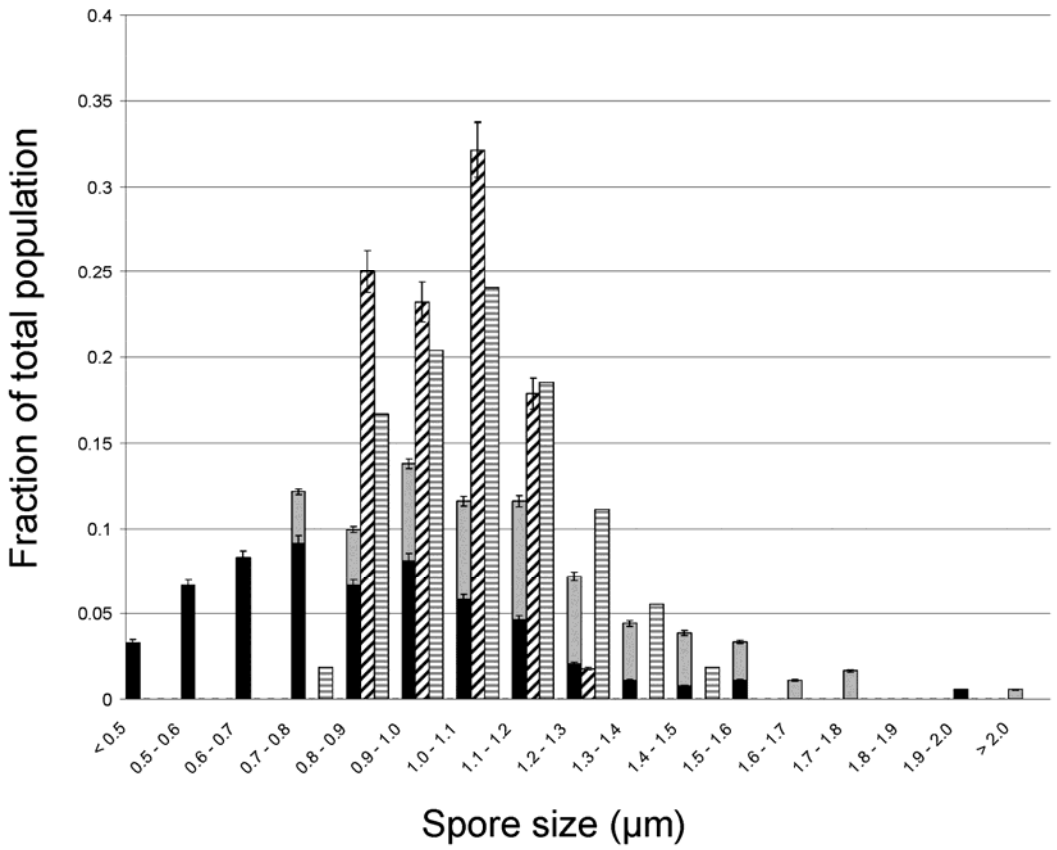

Figure S4. Statistical analysis of spore sizes. Bar diagrams of *S. coelicolor* M145 with control plasmid pSET152 (diagonal stripes), M145 with FtsZ expression construct pSCF7 (horizontal stripes) and the *ssgB* null mutant GSB1 harbouring pSCF7 (bar divided in black and grey shading, reflecting dead and viable spores respectively). The *ssgB* null mutant with control plasmid did not produce spores and is not included. Expression of FtsZ in the *ssgB* mutant restored some sporulation, but with a very broad distribution of spore sizes as compared to the wild-type strain. Most of the smaller sized spores in the *ssgB* mutant were nonviable (Fig. 4).

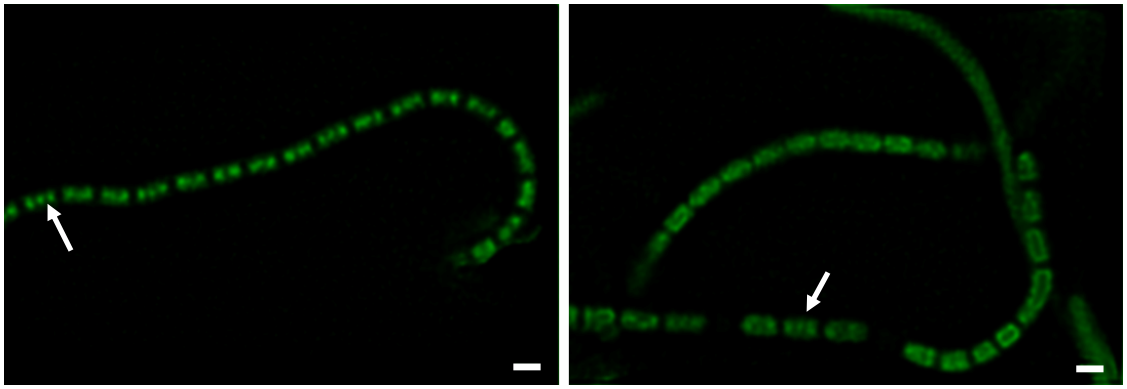

Fig S5. Deconvolution of fluorescence micrographs of spore chains of the *whiA* and *whiG* mutants harbouring plasmid pSCF7. **Left,** Δ*whiA* + pSCF7; **right,** Δ*whiG* + pSCF7. Several DNA lobes are observed in the immature spore chains in these strains, which were also observed with high-resolution TEM (see Fig. 5). In each image a typical multilobed spore is highlighted by an arrow. Bar, 1 μm. Deconvolution was performed with Huygens Professional 3.3.2, a PSF was extracted from a z-stack of 50 nm rotavirus particles coated with eGFP. This PSF was subsequently used to optimize the fluorescent images.

Table S1. Bacterial strains, plasmids and constructs used in this study.

Bacterial Strain	Description and/or genotype	Reference
E. coli strains		
JM109	*E.coli* K12 strain used for routine subcloning	(Sambrook *et al.*, 1989
ET12567	*E. coli* strain (StrR TetR CamR) that produces nonmethylated DNA that can be introduced into *S. coelicolor*	(Gust et al. 2004)
ET12567/pUZ8002	ET12567 containing plasmid pUZ8002 (KanR) that allows conjugative transfer of plasmids between *E. coli* and *Streptomyces*	(Gust et al. 2004)
Derivatives of S. coelicolor A3(2)		
M145	*S. coelicolor* A3(2) reference strain that lacks the natural plasmids SCP1 and SCP2	(Kieser et al. 2000)
FM145	derivative of M145 with reduced autofluorescence	(Willemse and van Wezel, 2009)
GSB1	M145 Δ*ssgB* (::*aacC4*)	(Keijser et al., 2003)
K202	M145 + pKF41	(Grantcharova et al. 2005)
Hu133	M145 *ftsZ*::*aph*	(McCormick et al. 1994)
J2400	M145 *whiG::hyg*	Flärdh *et al.*, 1999)
J2401	M145 *whiA::hyg*	Flärdh *et al.*, 1999)
J2402	M145 *whiB::hyg*	Flärdh *et al.*, 1999)
J2403	M145 *whiH::hyg*	Flärdh *et al.*, 1999)
J2450	M145 *whiI::hyg*	(Ainsa et al. 1999)
J2452	M145 *whiJ::hyg*	(Ainsa et al. 2010)
JSC18	J2400 + pSCF7	This study
JSC19	J2401 + pSCF7	This study
JSC20	J2402 + pSCF7	This study
JSC21	J2403 + pSCF7	This study
JSC22	J2450 + pSCF7	This study
JSC23	J2452 + pSCF7	This study

JSC24	GSB1 + pSCF7B	This study
JSC25	J2400 + pSCF1	This study
JSC26	J2401 + pSCF1	This study
JSC27	J2402 + pSCF1	This study
JSC28	J2403 + pSCF1	This study
JSC29	J2450 + pSCF1	This study
JSC30	J2452 + pSCF1	This study
JSC31	GSB1 + pSCF1	This study
Plasmids and constructs	**Description**	**Reference**
pHJL401	*Streptomyces*/*E. coli* shuttle vector (1-5 copies per genome in *Streptomyces*)	(Larson and Hershberger 1986)
pSET152	*Streptomyces/E. coli s*huttle vector that integrates at the φC31 attachment site (*att*P) in the *Streptomyces* genome	(Bierman et al. 1992)
pHM10a	*Streptomyces* integrative vector with *ermE* promoter and *S. ramocissimus tuf1* RBS. Integrates at the minicircle attachment site.	(Motamedi et al. 1995)
pSCF1	pHJL401 containing a 2.4 kb insert harbouring *ftsZ* and *ftsQ* expressed from their natural promoters	(van Wezel et al. 2000)
pSCF5	pHJL401 containing a 1.6 kb insert harbouring *ftsZ* expressed from its natural promoter	(van Wezel et al. 2000)
pSCF7	Integrative construct based on pSET152 expressing *S. coelicolor ftsZ* from the constitutive *ermE* promoter	(van Wezel et al. 2000)
pSCF7B	Derivative of pHM10a carrying the insert of pSCF7 and the *hyg* resistance cassette	this study

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