Figure S1. Removal of *mtrB* or replacement of MtrA with MtrA-3xFlag has no effect on *S. venezuelae* growth in liquid medium. A. Growth curves are *S. venezuelae* wild-type (blue), Δ*mtrB* mutant (red) and complemented mutant (green). Coloured arrows indicate the ChIP-seq time points. B. Growth rate in liquid MYM medium is also unaffected by replacing wild-type MtrA with MtrA-3xFlag in *S. venezuelae.*

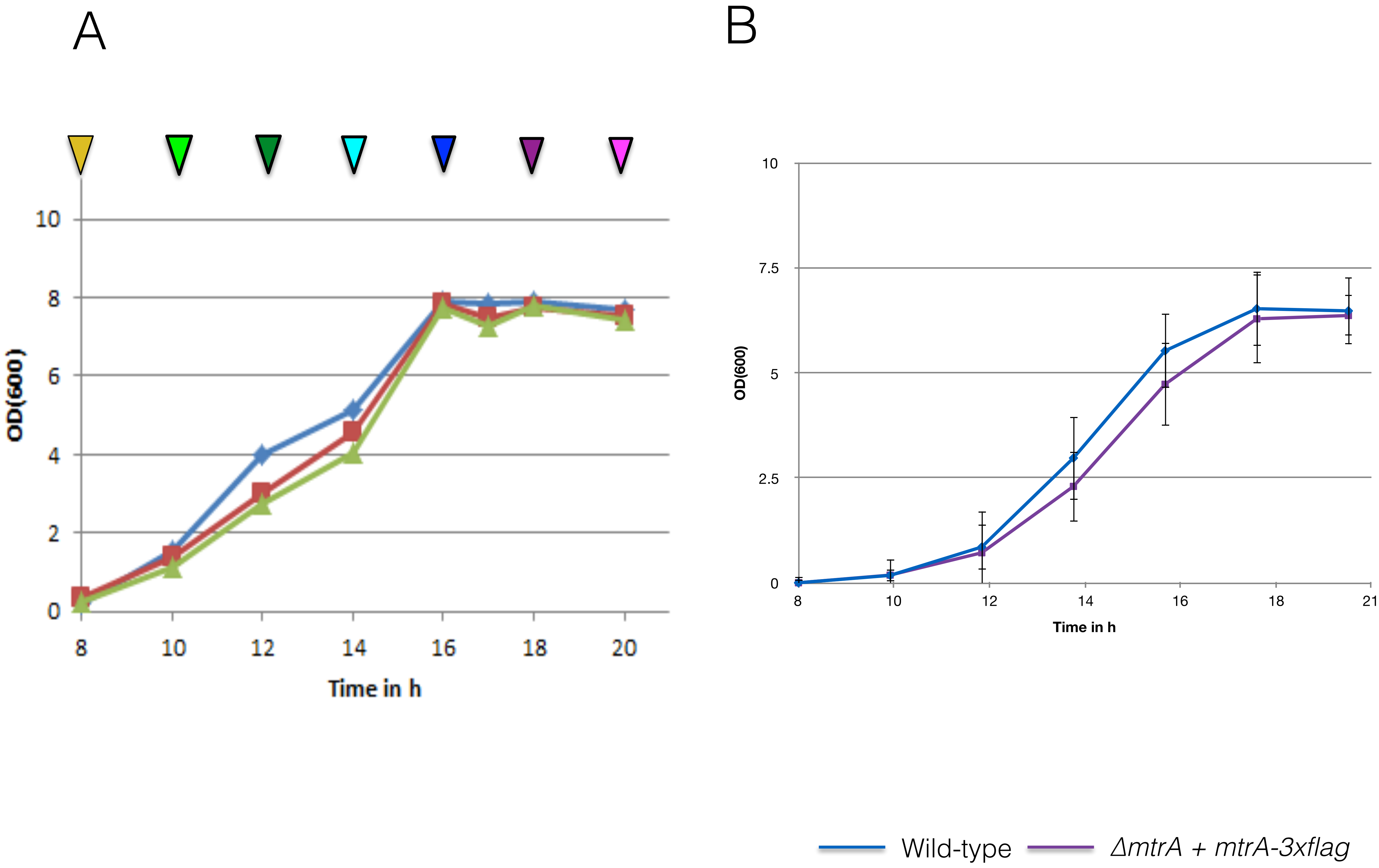


Figure S2. MtrA expression in *S. venezuelae* NRRL B-65442. A. Microarray data (EBI ArrayExpress E-MEXP-3612) shows a slight decrease in *mtrA* expression (green) following differentiation of substrate mycelium into aerial hyphae at 14 hours. This is consistent with ChIP- and RNA-seq data which shows that MtrA autoregulates and is active for DNA binding at 10 and 12 hours and down regulated from 14-18 hours. B. Immunoblotting with anti-Flag antibodies shows that MtrA-3xFlag is constitutively expressed from its own promoter. Control panel (S. ven, right) shows no cross reaction with anti-Flag antibodies in the wild-type. Coomassie stained SDS PAGE gels show equal protein loading for all samples.

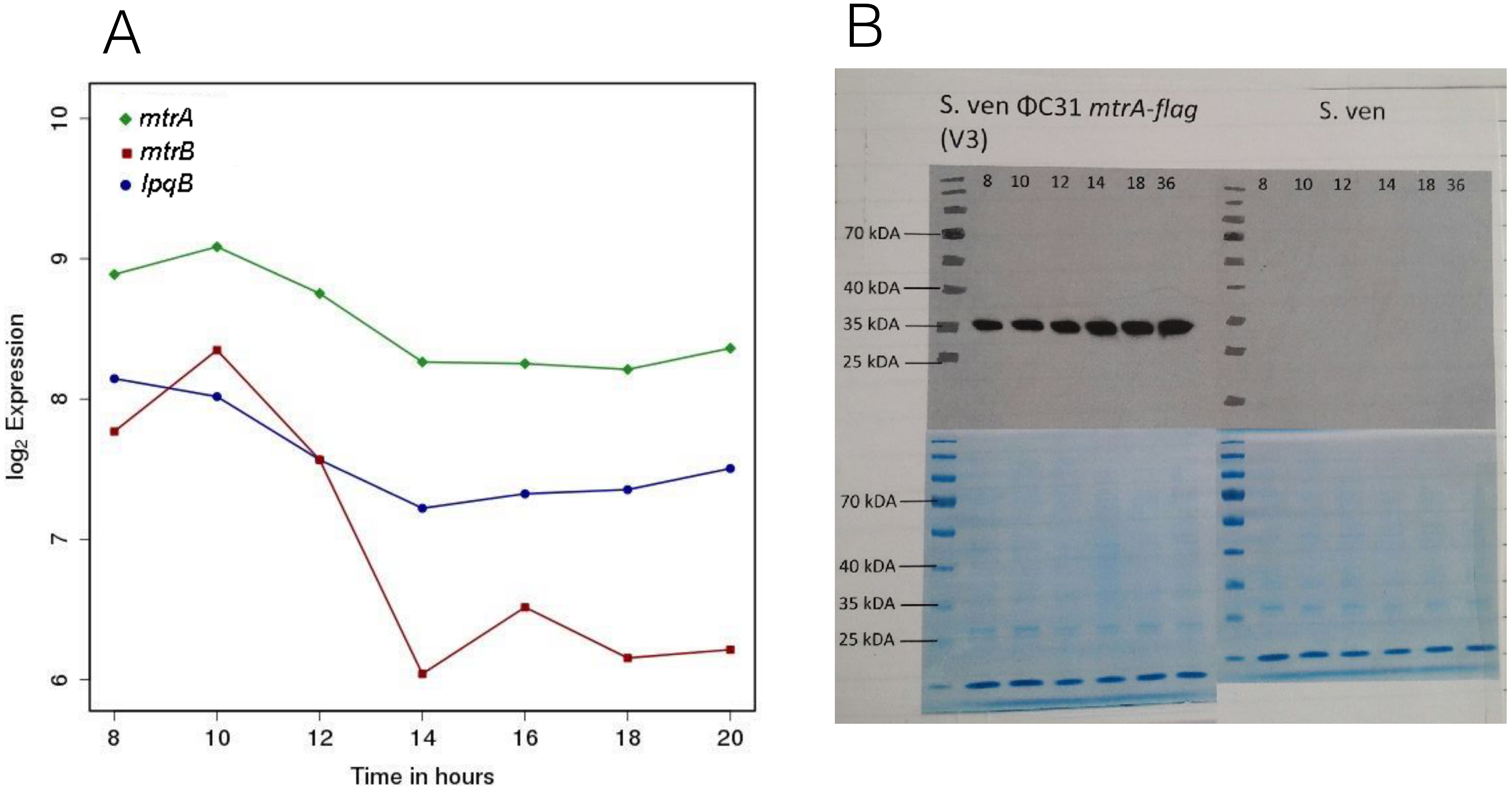


Figure S3. ChIP-seq during the *S. venezuelae life* cycle. The peaks show sequences enriched during ChIP-seq experiments against MtrA-3xFlag in *S. venezuelae* at 8, 10, 12, 14, 16, 18 and 20 hours with the wild-type control taken at 18 hours. See Figure S1 for more information about the different stages of growth used in this experiment.

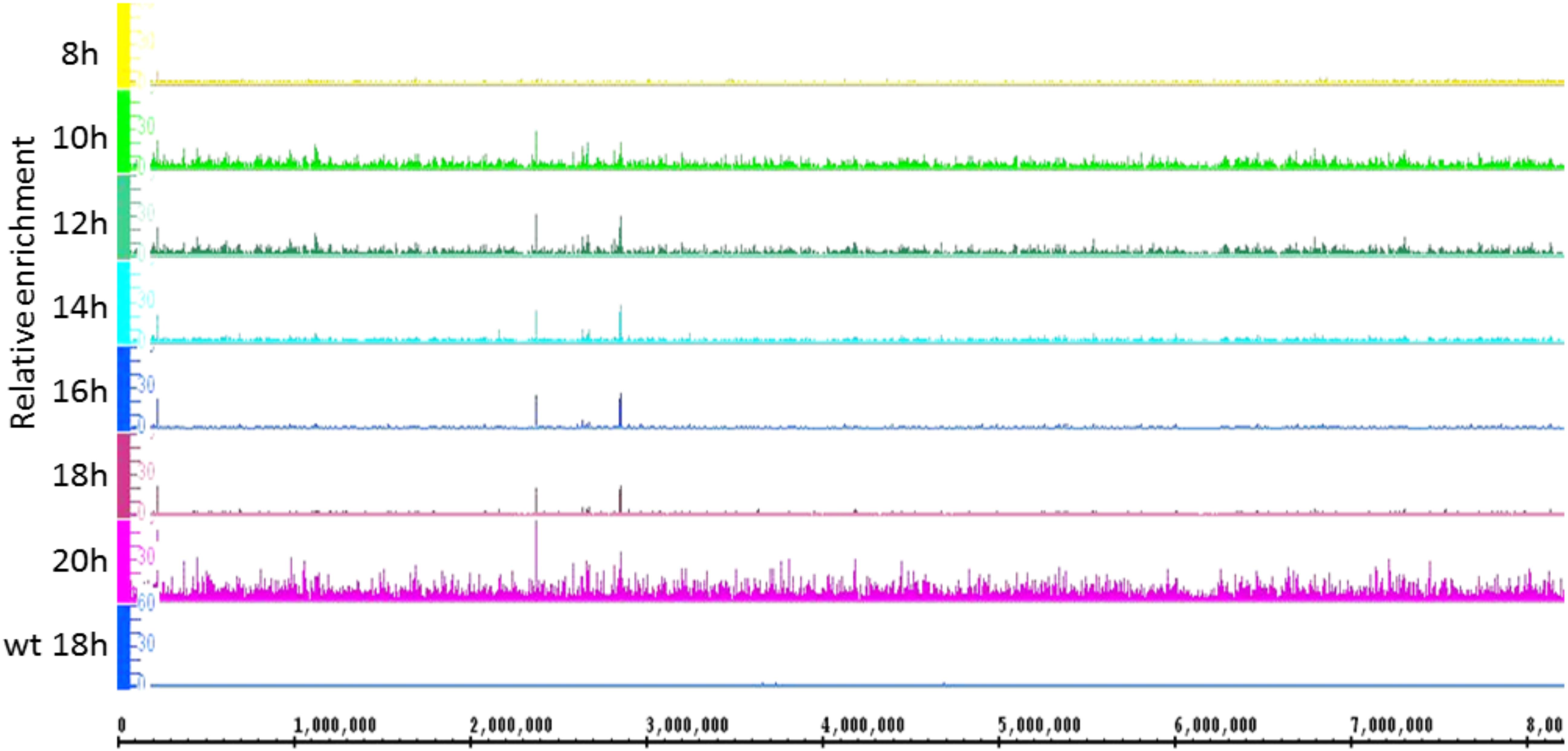


Figure S4. Deletion of *mtrB* switches on antibacterial activity in *S. venezuelae*. Left: Bioassays of *S. venezuelae* wild-type (WT), Δ*mtrB* and WT over-expressing *mtrA* from the high level *ermE** promoter (mtrA*) against *Bacillus subtilis* shows that only the Δ*mtrB* mutant has antibacterial activity The immunoblot (top) shows that MtrA is produced in all three strains. Right: Bioassay with filter paper disks soaked in methanol-extracted culture supernatants of the wild-type (WT) and Δ*mtrB* strains and chloramphenicol (Cm) or methanol (MeOH) controls. Zones of inhibition indicate antibacterial activity in the Δ*mtrB* extract and Cm control.

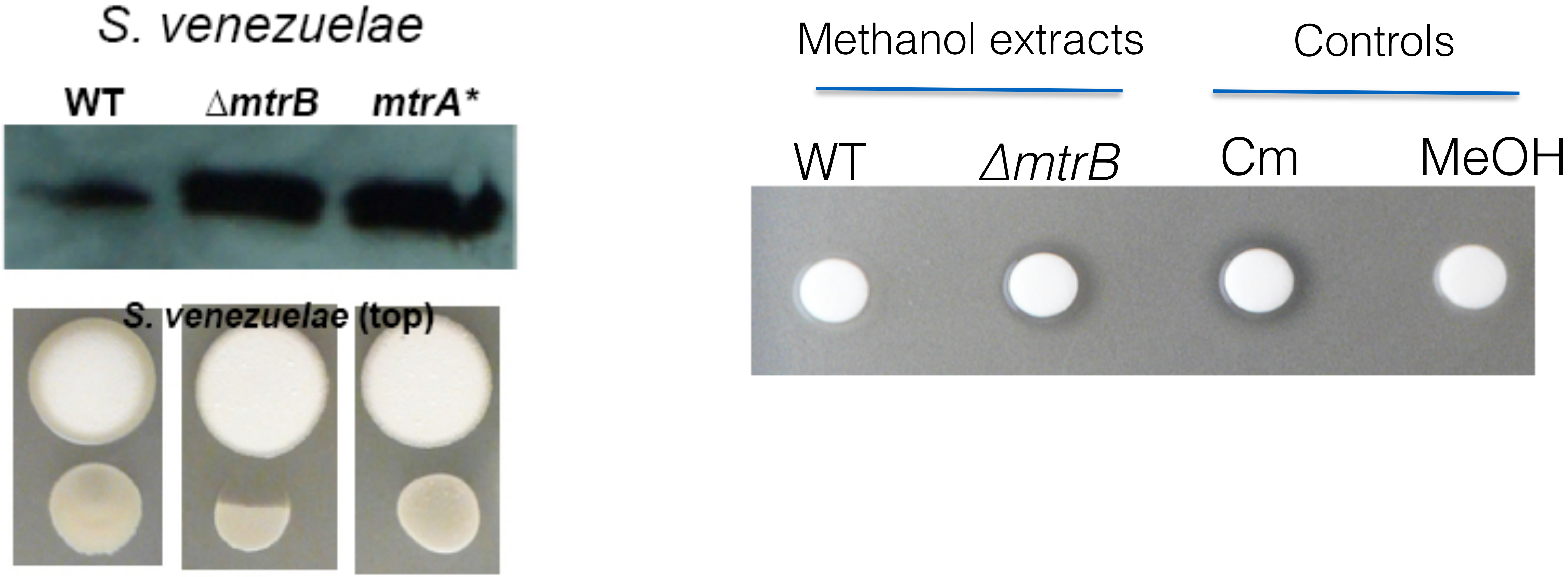


Figure S5. MtrA directly activates antibiotic production in *S. coelicolor*. ChIP-seq against MtrA-3xFlag in *S. coelicolor* shows that MtrA binds to the promoters of the cluster specific activator genes *actII-4* and *redZ* and the desferrioxamine repressor gene *desR*. The green line shows the wild type control, black is the 16 hour time point and blue is the 20 hour time point.

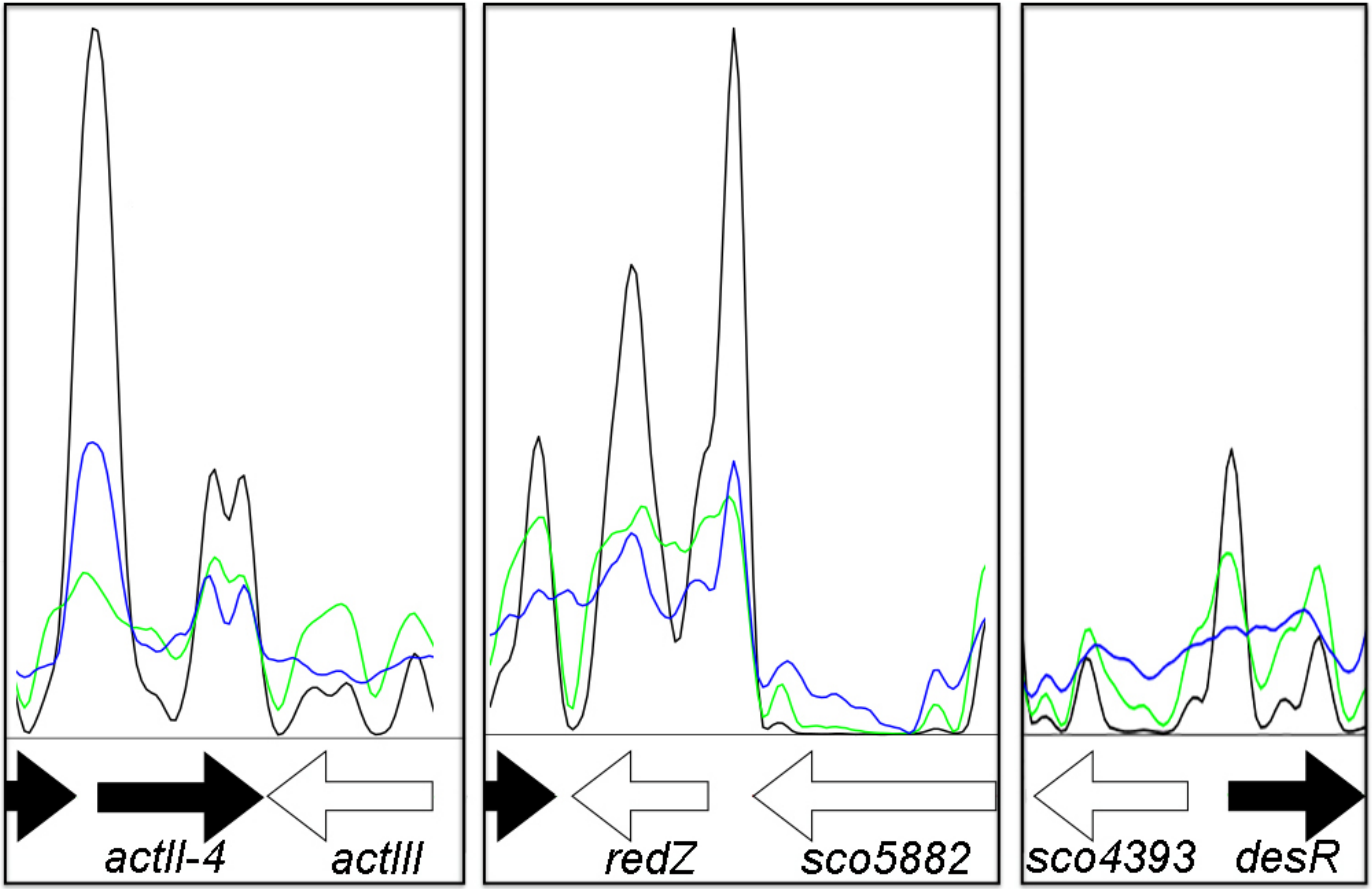
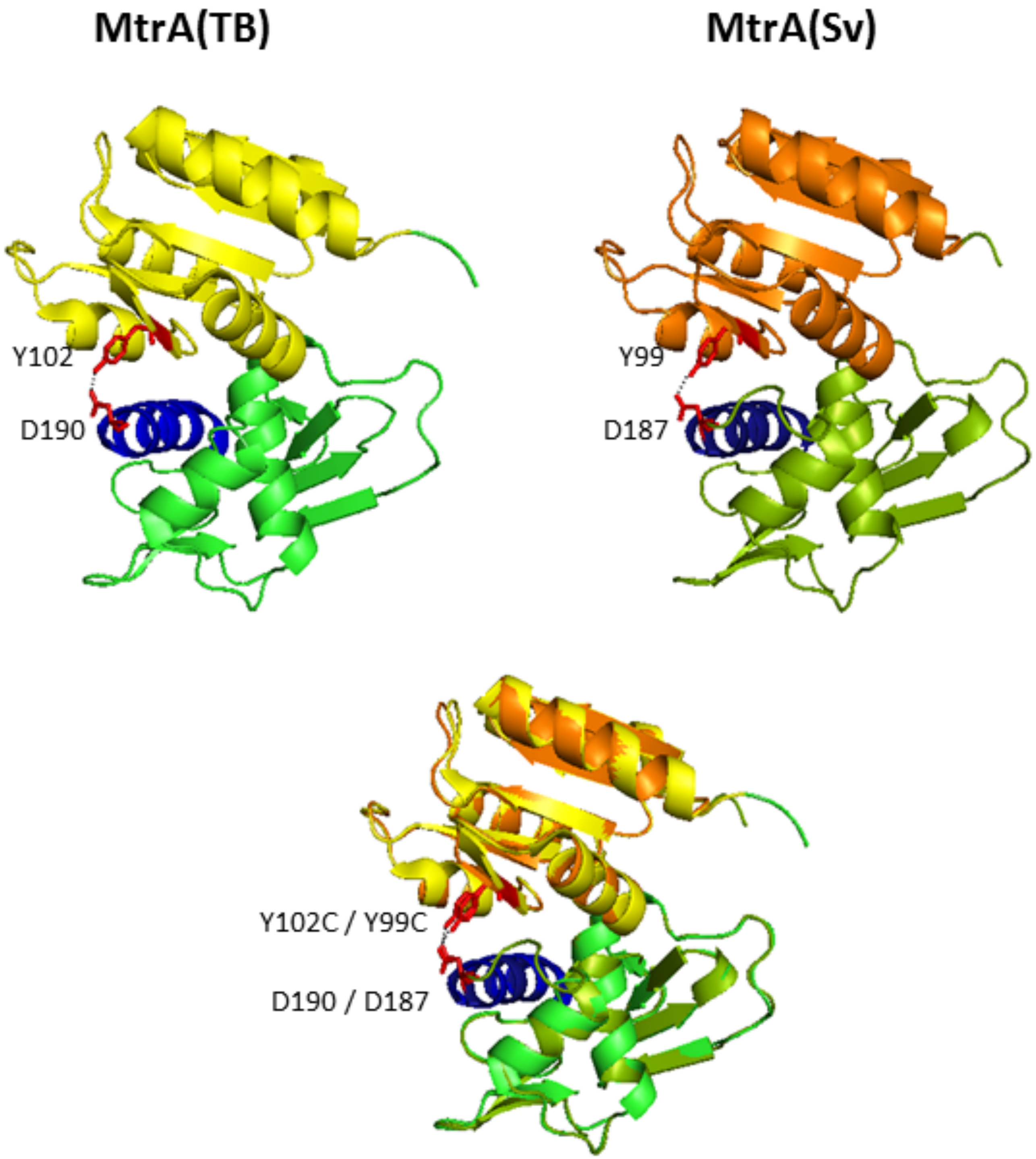


Figure S6. Structural modelling of Sv-MtrA. Left: Crystal structure of MtrA(TB). Right: the modelled structure of MtrA(Sv). Bottom: Overlay of MtrA(TB) and MtrA(Sv). The MtrA(TB) structure was obtained from the protein data bank (http://www.rcsb.org). Accesion number for MtrA(TB) is 2GWR. The structure of MtrA(Sv) was modelled with https://toolkit.tuebingen.mpg.de/hhpred and http://www.sbg.bio.ic.ac.uk/~phyre2. Both models were similar and for simplicity only the phyre2 model is shown.



MtrA(TB)

MtrA(Sv)

Overlay of MtrA(TB) and MtrA(Sv)

Table S2. ChIP-seq and RNA-seq data of target genes involved in development. An enrichment value in the ChIP-seq data below seven is not statistically significant. A fold change less than two fold is not statistically significant in the RNA-seq data.

				ChIP-seq									**RNA-seq**			
													wt	**ΔmtrB**	**14h**	**20h**
				SVEN						**SCO**	**strand**	**distance to TSS**	**14h compared to 20h**		**wt versus ΔmtrB**	
	SCO no.	**SVEN15 no.**	**Product**	**10**	**12**	**14**	**16**	**18**	**20**	**16**						
Genes required for formation of aerial hyphae																
bldD	SCO1489	SVEN15_1052	DNA binding transcription factor	1.7	1.5	0.5	0.1	0.3	1.7	6.0			-0.6	0.5	-0.5	0.6
c-di-GMP																
	SCO4931	SVEN15_4502	GGDEF domain protein	8	4.8	1.6	0.3	0.8	20	8.8	1	-42	**-4.8**	**0.5**	-4.4	0.5
cdgB	SCO4281	SVEN15_3942	GGDEF domain protein	4.8	3.5	1.5	0.4	0.4	10.7	8.3	1	-58	0.0	0.4	0.0	0.2
		SVEN15_0422	GGDEF / EAL domain protein	2.2	3	2.2	1.2	1.2	3.8	/	-1	-447	5.4	6.2	2.1	2.9
rmdB	SCO5495	SVEN15_5058	GGDEF / EAL domain protein	5.6	2.9	0.6	0	0.5	18.9	8.3	1	-465	-0.1	0.0	-0.3	-0.2
	SCO5511	SVEN15_5080	GGDEF / EAL domain protein	3.8	2.8	1.7	0.8	0.8	4.3	7.5	1	-160	**3.0**	**-0.1**	2.9	**-0.2**
Bald phenotype																
bldG	SCO3549	SVEN15_3247	**putative anti-anti sigma factor**	4.3	3.8	1.5			9.6	/	-1	79	-1.9	-1.0	-1.6	-0.7
adpA / bldH	SCO2792	SVEN15_2524	**DNA binding transcription factor**	2.4	3.5	1.6	0.5	0.9	5.8	/	1	-112	**-0.7**	**-3.6**	-0.6	**-3.6**

bldM	SCO4768	SVEN15_4355	**Two-component RR**	3.6	2.7	1.3	0.4	0.9	9.1	9.5	1	-257	**0.7**	**-2.4**	1.8	-1.3
bldN	SCO3323	SVEN15_3116	**ECF sigma factor**	2.9	2.1	0.6	0.1	1.1	12.3	7.3	1	-141	0.5	-0.9	1.2	-0.1
Genes required for sporulation																
whiD	SCO4767	SVEN15_4354	Putative transcription factor	3.8	2.7	1.3	0.4	0.9	10	9.5	-1	176	**8.3**	**10.6**	**-0.8**	**1.6**
wblE	SCO5240	SVEN15_4802		2.9	3.7	1.4	0.3	1.7	9.2	8.8	-1	33	**3.4**	**-0.1**	**2.1**	**-1.4**
wblM	SCO6922	SVEN15_5661											**3.0**	**-0.1**	**2.7**	**-0.4**
whiB	SCO3034	SVEN15_2717	Putative transcription factor	3.2	2.5	1	0.3	0.9	10.6	11.3	-1	135	**2.9**	**0.5**	**2.5**	**0.2**
sspA	SCO5321	SVEN15_6651											6.5	7.4	1.2	2.2
whiG	SCO5621	SVEN15_5191	Sigma factor	5.2	3.4	1.6	0.9	1.1	5	/	1	-253	**2.0**	**0.1**	**2.6**	**0.8**
whiH	SCO5819	SVEN15_5388	GntR-like transcription factor	3.3	2.9	1.3	0.2	1	12	7.6	1	41	**2.0**	**0.1**	**2.6**	**0.8**
whiI	SCO6029	SVEN15_5706	Two-component RR	8.4	5.6	2.7	0.8	1.3	20.6	17.6	-1	137	**5.7**	**2.2**	**4.0**	**0.4**
ssgA	SCO3926	SVEN15_3615	putative regulator	1.3	1	0.4		0.9	8.3	7.6	1	-79	**0.3**	**-2.1**	**2.0**	**-0.4**
ssgB	SCO1541	SVEN15_1102	putative regulator	5.1	3.3	1.9	1.2	1.2	11.3	/	1	-111	**7.2**	**4.2**	**4.3**	**1.3**
ssgD	SCO6722	SVEN15_6261	putative regulator	2.6	2.8	1.3	0.6	0.4	2.8	/	-1	27	**0.5**	**-3.9**	**2.5**	**-1.9**
ssgE	SCO3158	SVEN15_2937	putative regulator	4.9	3.5	1.2	0.3	0.5	15.3	8.2	1	-70	1.5	0.8	1.9	1.3
ssgG	SCO2924	SVEN15_2622	putative regulator	8.7	4.7	1.7	0.5	0.9	6.4	/	1	-182	**4.2**	**-0.9**	**5.1**	**0.1**
Hydrophobic sheath														0.0	0.0	

chpC	SCO1674	SVEN15_1231											2.1	-2.4	**3.9**	**-0.6**
chpH	SCO1675	SVEN15_1232	Hydrophobic cell wall-associated proteins	3.4	1.6	0.5		0.6	8.6	/	-1	57	**3.1**	**-0.9**	**3.4**	**-0.6**
chpE	SCO1800	SVEN15_1396		0.4	0.8	0.6		0.3	1.4	/	-1	49	-1.0	-4.9	2.8	-1.0
chpG	SCO2699	SVEN15_4550		2.3	2.8	1	1.8	1.2	5.6	/	1	55	**3.8**	**1.7**	**3.0**	**0.9**
chpF	SCO2705	SVEN15_4546		2.8	2.3	2	1	1.6	7.8	14.1	1	-183	**7.8**	**4.1**	**5.2**	**1.5**
chpD	SCO2717	SVEN15_4533		3.2	2.7	1	0.5	1.1	7.5	/	1	-40	2.9	1.8	2.8	1.7
sapB	SCO6682	SVEN15_6339	Lantibiotic-like peptide surfactant	6.8	5.2	2.3	1	0.7	8.1	14.7	-1	-12	**3.3**	**-2.2**	**2.5**	**-3.0**
rdlA	SCO2719	SVEN15_4528	Secreted cell wall-associated protein	0.2	0.6	0.6	0.3	0.5	1	/	-1	60	**5.8**	**1.3**	**3.0**	**-1.5**
rdlB		SVEN15_4531		1.8	1.2	0.9	0.7	0.4	1.5	/	-1	46	**4.5**	**1.2**	**3.2**	**0.0**
rdlA		SVEN15_4532		1.4	1.4	0.7	0.4	0.7	2.9	/	1	-21	**3.8**	**1.2**	**2.9**	**0.3**
Cell division initiation																
dnaN / oriC	SCO3878	SVEN15_3570	DNA polymerase III / origin of replication	3.1	1.5	0.4		0.6	6.3	6.7	-1	235	0.0	-0.8	0.2	-0.6
				1.2	2.3		0.1	0.7	8	/	-1	611				
dnaA	SCO3879	SVEN15_3571	Chromosome replication initiation protein	0.7	0.9				4.5	/	-1	125	0.6	0.6	0.4	0.4
Cell division																
ftsZ	SCO2082	SVEN15_1692	Tubulin-like cell division protein	4.8	4	1.5	0.5	0.6	15.5	10.2	-1	165	0.7	0.0	1.2	0.5
ftsI	SCO2090	SVEN15_1700	Penicillin binding protein										**2.5**	**1.2**	**2.6**	**1.3**

divIVA	SCO2077	SVEN15_1687	Tip-associated protein	1	2	1	0.5	0.5	8	/	-1	47	**1.1**	**0.2**	**0.0**	**-1.0**
filP	SCO5396	SVEN15_4942	Filament protein	3.2	2.4	1	0.4	0.5	9.8	11.5	-1	57	**-0.2**	**-1.2**	**-1.3**	**-2.3**
smeA	SCO1415	SVEN15_0971	Small membrane protein	5.8	4.9	1.6	0.6	1.7	21.5	15.6	1	23	7.4	8.5	1.5	2.6
sffA	SCO1416	SVEN15_0972	Putative DNA translocase										6.3	7.8	1.8	3.3
smc	SCO5577	SVEN15_5163	Chromosome-associated ATPase	3.2	2.5	1.1	0.9	0.9	2.4	8.4			3.8	4.5	0.0	0.7
mtrAB-lpqB																
mtrA	SCO3013	SVEN15_2696	Two component RR	10	7.7	2.4	1.2	1.4	19.4	6.0	-1	42	-0.2	0.1	2.9	3.2

Table S3. Biosynthetic gene clusters (BGCs) predicted by antiSMASH in *S. venezuelae* and MtrA regulation of the cluster. BGCs coloured in amber are bound by MtrA and the regulation is not known or individual genes are positively and negatively regulated, green BGCs are positively regulated, red BGCs are negatively regulated and yellow BGCs are not bound by MtrA. N/S: Not significant.

BGC	**Product**	**SVEN15 Gene no.**	**ChIP target**	**RNA-seq LogFC WT/ΔmtrB**	**MtrA regulation**
1	Ectoine	0205-8	0205	N/S	Not known
2	Unknown (PKS/NRPS)	0448-72	0450	1.24 (14h)	Positive
			0451	N/S	Not known
3	Thiazostatin	0473-85	0473	N/S	Not known
			0475	-0.97 (14h)	Negative
4	Unknown (Lantibiotic)	0506-18	0512	N/S	Not known
			0513	N/S	Not known
			0514	-3.18 (20h)	Negative
			0515	-3.43 (20h)	Negative
5	Unknown (Lantibiotic)	0585-91	0585	N/S	Not known
			0591	-1.18 (14h)	Negative
6	Arcyriaflavin	0724-34	0725	N/S	Not known
			0731	N/S	Not known
			0732	-1.07 (14h)	Negative
7	Chloramphenicol	0877-94	0879	N/S	Not known
			0878	-1.59 (14h)	Negative
			0880	3.01 (20h)	Positive
8	Unknown	1792-832	1802	-0.82 (14h)	Negative
			1815	N/S	Not known
			1820	N/S	Not known
			1821	N/S	Not known
			1828	-1.82 (14h)	Negative
			1830	N/S	Not known
9	Desferrioxamine	2514-7	No	N/S	None
10	Unknown (Lassopeptide)	3042-65	3043	N/S	Not known
11	Unknown	3973-4016	4014	N/S	Not known
			4016	1.15 (20h)	Positive
12	Unknown (Butyrolactone)	4085-95	4093	N/S	Not known
			4094	-1.49 (14h)	Negative
			4095	-1.79 (14h)	Negative
13	Melanin	4547-57	4548	0.979 (14h)	Positive
			4556	N/S	Not known
			4557	0.991 (20h)	Positive
14	Unknown (Butyrolactone)	4983-99	4996	-2.033	Negative

🟥			4998	N/S	Not known
🟩 15	Unknown (RiPP)	5018-38	5028	0.76	Positiv
			5029	N/S	Not known
🟧 16	Flaviolin (predicted)	5241-73	5265	N/S	Not known
🟩 17	Unknown (Siderophore)	5304-15	5307	N/S	Not known
			5308	1.35	Positive
			5311	N/S	Not known
🟧 18	Unknown (Siderophore)	5361-72	5362	N/S	Not known
🟧 19	Unknown (Bacteriocin)	5709-19	5709	N/S	Not known
			5712	N/S	Not known
			5714	2.4 (14h)	Positive
			5717	-2.1 (14h)	Negative
🟧 20	Jadomycin	5847-73	5858	N/S	Not known
			5861	N/S	Not known
			5863	N/S	Not known
			5868	N/S	Not known
🟥 21	Unknown (NRPS)	5995-6029	5995	N/S	Not known
			6000	N/S	Not known
			6008	N/S	Not known
			6009	N/S	Not known
			6010	-2.09 (14h)	Negative
			6012	N/S	Not known
🟥 22	Unknown (NRPS)	6050-77	6052	-1.72 (14h)	Negative
			6053	-0.79 (14h)	Negative
			6063	-0.92 (20h)	Negative
🟧 23	Unknown (NRPS)	6101-25	6101	N/S	Not known
			6108	-0.89	Negative
			6109	N/S	Not known
			6111	1	Positive
			6120	-1.19	Negative
			6124	0.88	Positive
			6125	N/S	Not known
🟧 24	Hopanoids	6296-6320	6296	N/S	Not known
			6297	2.15 (14h)	Positive
			6311	0.84 (20h)	Positive
			6315	N/S	Not known
			6316	N/S	Not known
			6317	-3.17	Negative
🟩 25	Unknown (Bacteriocin)	6385-6393	6385	1.31	Positive
			6390	1.79	Positive

26	Spore pigment	6643-6652	No	All genes >1 in ΔmtrB except 6652 at 20h	Indirect, positive
27	Melanin	6688-6695	6688	-0.9	Negative
			6689	-3.41	Negative
28	Unknown (siderophore; NRPS)	6883-6907	6901	N/S	Not known
			6904	N/S	Not known
29	2-methylisoborneol	6950-67	6951	0.971 (20h)	Positive
			6964	N/S	Not known
			6966	2.65 (14h)	Positive
			6968	N/S	Not known
30	Venemycin	7070-103	7074	1.27 (14h)	Positive
			7080	N/S	Not known
			7083	N/S	Not known
			7092	0.86 (14h) / 1.40 (20h)	Positive
			7103	N/S	Not known
31	Unknown	7258-63	No	N/S	None

Table S4. BGCs in *S. coelicolor* and genes within the cluster bound by MtrA. Green indicates there is at least one MtrA target gene in the BGC, red indicates no MtrA binding sites and amber indicates indirect regulation by MtrA as judged by LCMS (BGCs 9 and 26, Figure 6) and ChIP (BGC 9, MtrA binds upstream of *desR* which is situated outside this BGC but represses its expression, see Figure S9).

BGC	Product	Genes	ChIP target	Reference
🟩 1	Eicosapentaenoic acid (predicted)	*SCO0124-29*	*SCO0126, 27*	[1]
🟩 2	Isorenieratene (Carotenoid)	*SCO0185-91*	*SCO0185*	[1]
🟩 3	Unknown (Lantipeptide)	*SCO0247-0279*	*SCO0249, 50, 52, 53, 61, 68, 75*	*AntiSMASH* [2]
🟩 4	Coelichelin	*SCO0489-99*	*SCO0489, 90*	[1]
🟥 5	Unknown (Bacteriocin)	*SCO0750-756*	/	*AntiSMASH* [2]
🟩 6	Flaviolin	*SCO1185-1226*	*SCO1185, 87, 95, 1200, 01, 09, 10*	*AntiSMASH* [2]
🟩 7	Ectoine	*SCO1864-67*	*SCO864*	*AntiSMASH* [2]
🟩 8	Melanin	*SCO2693-2707*	*SCO2705, 06*	*AntiSMASH* [2]
🟨 9	Desferrioxamine	*SCO2782-85*	/	[1]
🟩 10	Calcium Dependent Antibiotic	*SCO3210-3249*	*SCO3229, 30, 32, 34, 36, 41*	[1]
🟩 11	Actinorhodin	*SCO5071-92*	*SCO5082, 83, 85*	[1]
🟩 12	Albaflavenone	*SCO5212-5231*	*SCO5217*	[3]
🟥 13	Spore pigment	SCO5314-5320	/	[1]
🟩 14	Unknown (Siderophore)	SCO5799-5801	*SCO5801*	[1]
🟩 15	Undecylprodigiosin	SCO5877-5898	*SCO5881, 83, 86*	[1]
🟩 16	Unknown (Bacteriocin)	SCO6041-52	*SCO6042, 44, 49, 50*	*AntiSMASH* [2]
🟩 17	Geosmin	SCO6064-81	*SCO6080*	*AntiSMASH* [2]
🟩 18	Unknown (Siderophore)	SCO6221-32	*SCO6222, 23, 26, 27*	*AntiSMASH* [2]
🟩 19	Coelimycin (Cpk)	SCO6273-88	*SCO6273, 80*	[1,3]

🟥 20	Unknown (NRPS)	SCO6429-38	/	[3]
🟩 21	SapB	SCO6668-87	*SCO6671, 75, 76, 83*	*AntiSMASH* [2]
🟩 22	Hopanoids	SCO6759-71	SCO6772	[3]
🟥 23	Unknown (PKS)	SCO6826-27	/	[1]
🟥 24	Unknown (Lantipeptide)	SCO6921-43	/	AntiSMASH [2]
🟩 25	Unknown	SCO7176-7200	*SCO7176, 77*	AntiSMASH [2]
🟨 26	Germicidin	SCO7221	/	[3]
🟩 27	Unknown	SCO7454-77	*SCO7462, 63, 68*	AntiSMASH [2]
🟩 28	Coelibactin	SCO7681-91	*SCO7681, 82, 84, 87*	[1]
🟥 29	2-methylisoborneol	SCO7700-1	/	[3,4]

References.

1. Bentley, S. D. *et al.* Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). *Nature* **417,** 141–147 (2002).
2. Weber, T. *et al.* antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* **43,** W237–43 (2015).
3. Challis, G. L. Exploitation of the Streptomyces coelicolor A3(2) genome sequence for discovery of new natural products and biosynthetic pathways. *J Ind Microbiol Biotechnol* (2013). doi:10.1007/s10295-013-1383-2
4. van Keulen, G. & Dyson, P. J. in **89,** 217–266 (Elsevier, 2014).

Table S6 Bacterial strains

Strain	Description	Plasmid	Resistance	Reference
E. coli				
Top10	F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G			Invitrogen^{TM}
BW25113	λ^{-}, Δ(*araD-araB*)*567*, Δ*lacZ4787*(*::rrnB-4*), *lacIp-4000*(*lacIQ*), *rpoS369*(*Am*), *rph-1*, Δ(*rhaD-rhaB*)*568*, *hsdR514*	pIJ790	Cml^{R}	[1]
ET12567	*dam*$^{-}$ *dcm*$^{-}$ *hsdS*$^{-}$	pUZ8002	Cml^{R}/Tet^{R}	[1]
BL21 *λ*DE3	*fhuA2 [lon] ompT gal (λ DE3) [dcm] ∆hsdSλ DE3 = λ sBamHIo ∆EcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 ∆nin5*			
S. coelicolor				
M145	SCP1^{-} SCP2^{-} Pgl+			[2]
ΔmtrB	M145 *mtrB::scar*			This work
NS0171	M145 *mtrA::scar* ΦBT1 *mtrAp mtrA*-3xFlag	pNS109		This work
S. venezuelae				
S. venezuelae ATCC 10712	Wild-type			John Innes Centre, Norwich
NS003	ΦBT1 *mtrAp mtrA*-3xFlag	pNS109	Hyg^{R}	This work
NS012, NS021 and NS022	*mtrB*::*aac(3)IV oriT*		Apr^{R}	This work

NS013	ΦBT1 *mtrAp mtrA*(TB)	pNS103	Hyg^R	This work
NS016	ΦBT1 *mtrAp mtrA*(TB)$_{Y102C}$	pNS104	Hyg^R	This work
NS029	ΦBT1 pMS82	pMS82	Hyg^R	This work
NS033	ΦBT1 *ermE* mtrA*	pNS102	Hyg^R	This work
NS036	ΦBT1 pIJ10257	pIJ10257	Hyg^R	This work
NS039	ΦBT1 *mtrAp mtrA*	pNS108	Hyg^R	This work
NS042	*mtrA*::*aac(3)IV oriT* ΦBT1 *mtrAp mtrA*-3xFlag	pNS109	Apr^R / Hyg^R	This work
NS052	*mtrA*:: *aac(3)IV oriT* ΦBT1 *mtrAp mtrA*	pNS108	Apr^R / Hyg^R	This work
NS093	*mtrB*:: *aac(3)IV oriT* ΦBT1 *mtrAp mtrB*	pNS107	Apr^R / Hyg^R	This work
NS099	ΦBT1 *mtrAp mtrA*$_{Y99C}$	pNS105	Hyg^R	This work

Table S7. Plasmids and cosmids used in this work

Plasmids	**Genotype/description**	**Resistance**	**Reference**
pIJ773	*aac(3)IV oriT bla*	Apr^R	[1]
pIJ790	*araC-Parab,Y, β, exo, cat, repA1001ts, oriR101*	Cml^R	[1]
pUZ8002	RK2 derivative with a mutation in *oriT*	Kan^R	[1]
pMS82	*ori,* pUC18, *hyg, oriT,* RK2, int ΦBT1	Hyg^R	[3]
pIJ10770	*ori,* pUC18, *hyg, oriT,* RK2, int ΦBT1 Δ *aac(3)IVp*	Hyg^R	S Schlimpert, unpublished
pIJ10257	*oriT*, ΦBT1 *attB-int*, Hygr, *ermEp**, pMS81 backbone	Hyg^R	John Innes Centre, Norwich
pNS074	pETduet-1 *mtrA*(Sv)	Amp^R	This work
pETduet-1	f1 origin, T7 *lac*, *lacI, ori* with two multiple cloning sites	Amp^R	Novagen^{TM}
pNS100	pETduet-1 *mtrA* His-Cterm	Kan^R	This work
pNS102	pIJ10257 *mtrA*	Hyg^R	This work
pNS103	pIJ10770 *mtrAp mtrA*(TB)	Hyg^R	This work

pNS104	pIJ10770 *mtrAp mtrA*(TB)$_{Y102C}$	Hyg^R	This work
pNS105	pIJ10770 *mtrAp mtrA*(Sv)$_{Y99C}$	Hyg^R	This work
pNS106	pMS82 *mtrAp lpqB*	Hyg^R	This work
pNS107	pIJ10770 *mtrAp mtrB*	Hyg^R	This work
pNS108	pMS82 *mtrAp mtrA*	Hyg^R	This work
pNS109	pIJ10770 *mtrAp mtrA*-3xFlag	Hyg^R	This work
Cosmids			
SV-6-A04	Supercos-1 + 40.2kbp chromosomal fragment containing *mtrAB-lpqB*		John Innes Centre, Norwich
pNS069	SV-6-A04 *lpqB*:: *aac(3)IV oriT*		This work
pNS070	SV-6-A04 *mtrB*:: *aac(3)IV oriT*		This work
pNS071	SV-6-A04 *mtrA*:: *aac(3)IV oriT*		This work

Table S8. Primers used in this work

Primer	**Description**	**Sequence**
NS-040	*mtrA* (SVEN15_2696) forward disruption primer (Redirect)	GCCTTGAGACTGATACGGAAATGGGATGATGTCGATATGATTCCGGGGATCCGTCGACC
NS-041	*mtrA* (SVEN15_2696) reverse disruption primer (Redirect)	TCCCCGGGCTTCGGAGCAGCACTGCCTGTACTCATGTCATGTAGGCTGGAGCTGCTTC
NS-044	*mtrB* (SVEN15_2695) forward disruption primer (Redirect)	TCCGTGGTGTCGGGTACAAGGCGGACCGAGCTGACATGATTCCGGGGATCCGTCGACC
NS-080	*mtrB* (SVEN15_2695) reverse disruption primer (Redirect)	CTCCCGCACCCACCGTCCCGCTCGGGCGTCAGCGCCCACTGTAGGCTGGAGCTGCTTC
NS-131	*mtrA* (SVEN15_2696) forward test primer -141	TGA CAT CCA TGT CTG GCA TCA ACA CCC A
NS-132	*mtrA* (SVEN15_2696) reverse test primer -120	CGA CGA TCC TCA GCT GGA TGT TCC GT
NS-070	*mtrB* (SVEN15_2695) forward test primer -100	CAC GTC CAG CGG CTG CGC TCG AAG G
NS-071	*mtrB* (SVEN15_2695) reverse test primer -100	GTC GGG CAT GGT CGC GCA CCC GGT G
P1	*aac(3)IV* resistance cassette test primer	ATT CCG GGG ATC CGT CGA CC
P2	*aac(3)IV* resistance cassette test primer	TGT AGG CTG GAG CTG CTT C

NS-064	pMS82 forward test primer	GCA ACA GTG CCG TTG ATC GTG CTA TG
NS-065	pMS82 reverse test primer	GCC AGT GGT ATT TAT GTC AAC ACC GCC
NS-115	*mtrA* (SVEN15_2696) NdeI forward	GCGCATATGATGAAGGGACGCGTT
NS-116	*mtrA* (SVEN15_2696) HindIII reverse	GCGAAGCTTTCAGCTCGGTCCCGC
NS-121	pIJ10257 forward test primer	AGGTACCAGCCCGACCCG
NS-122	pIJ10257 reverse test primer	ATCAGCGAGCTGAAGAAA
NS-123	*mtrA* (SVEN15_2696) HindIII forward 1	GCGAAGCTTCCG TGG GCC GGT CCC GCC TC
NS-124	*mtrA* (SVEN15_2696) KpnI reverse	GCGGGTACCTCAGCTCGGTCCCGCCTTGT
NS-125	*mtrB* (SVEN15_2695) reverse 2	CGGAGCAGCACTGCCTGTACTCATATCGACATCATCCCATTT
NS-126	*mtrB* (SVEN15_2695) forward 3	ATGAGTACAGGCAGTGCTGCTCCGAAGCCC
NS-127	*mtrB* (SVEN15_2695) NsiI reverse 4	GCGATGCATTCAGCGCCCACGTGTCCTGT
NS-145	*mtrA* (SVEN15_2696) forward BamHI (pETduet)	GGATCCTAAAGGCCGCGTTCTGGTCGTC
NS-146	*mtrA* (SVEN15_2696) reverse HindIII (pETduet)	AAGCTT TCAGCTCGGTCCCGCCTTGTACC
NS-160	*mtrB* (SVEN15_2695) forward internal test primers	TTCAACGTGGTCGCGCTCTCCCTCG
NS-161	*mtrB* (SVEN15_2695) reverse internal test primers	GCCAGCTTCACCACGACGTCCTTGC
NS-166	pET28a(+) forward test primers	CTCGATCCCGCGAAATTAATACGAC
NS-167	pET28a(+) reverse test primers	GGGCTTTGTTAGCAGCCGGATC
NS-283	pSS170 forward test primer	CGGGGTCTGACGCTCAGTGGAACGAAA
Universal M13-6FAM Labelled Primers	Forward	GTAAAACGACGGCCAGT
	Reverse	CAGGAAACAGCTATGAC
NS-176	M13FAM nested SVEN15_ 0205p (*ectA*)forward	CTA AAA CGA CGG CCA GTC ACC GAA CGG AGC CGG GC
NS-177	M13FAM nested SVEN15_ 0205p (*ectA*)reverse	CAG GAA ACA GCT ATG AGG GCG CCG AAG GTA CCG A
NS-286	M13FAM nested SVEN15_3571p (*dnaA*) forward	**CTA AAA CGA CGG CCA GT**C GGT TCG CAA GGA TGG CTC GGC

NS-287	M13FAM nested SVEN15_3571p (*dnaA*) reverse	**CAG GAA ACA GCT ATG AC**T GCG GCA AGA TCA GCA GGA ACG TCA
NS-294	M13FAM nested SVEN15_0880p (*cmlR2*) forward	**CTA AAA CGA CGG CCA GT**G AAA AAG CTC CAA CTA CAT CGC AGA
NS-295	M13FAM nested SVEN15_0880p (*cmlR2*) reverse	**CAG GAA ACA GCT ATG AC**C CAT CAT GAT CAT GGC TGT CTG GTG

References

1. Gust, B., Challis, G. L., Fowler, K., Kieser, T. & Chater, K. F. (2003). PCR-targeted Streptomyces gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci USA* **100,** 1541–1546.
2. Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F. & Hopwood, D. A. (2000) Practical *Streptomyces* Genetics, John Innes Centre, Norwich, UK.
3. Gregory, M. A., Till, R. & Smith, M. C. M. (2003) Integration site for Streptomyces phage phiBT1 and development of site-specific integrating vectors. *J Bacteriol* **185,** 5320–5323.