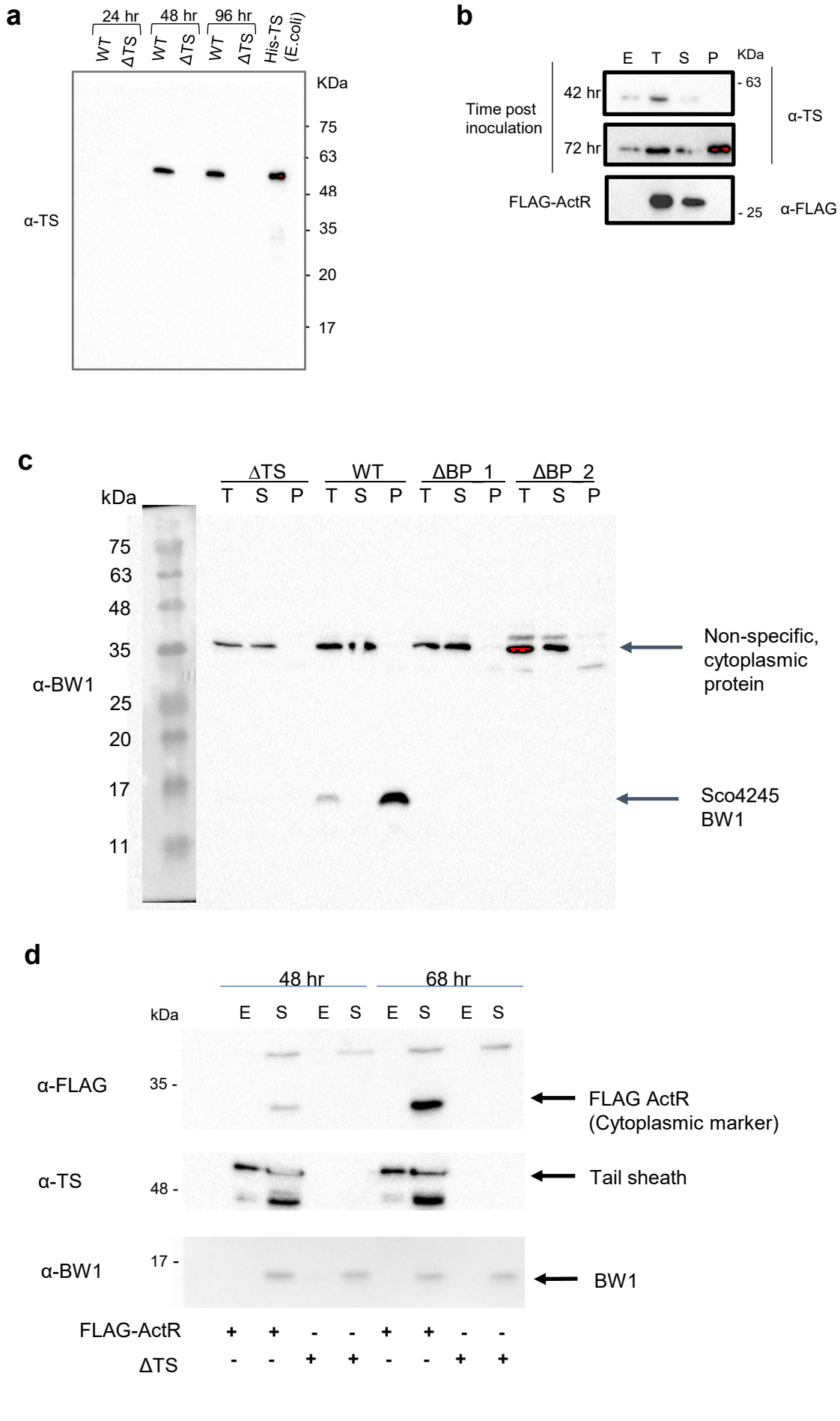
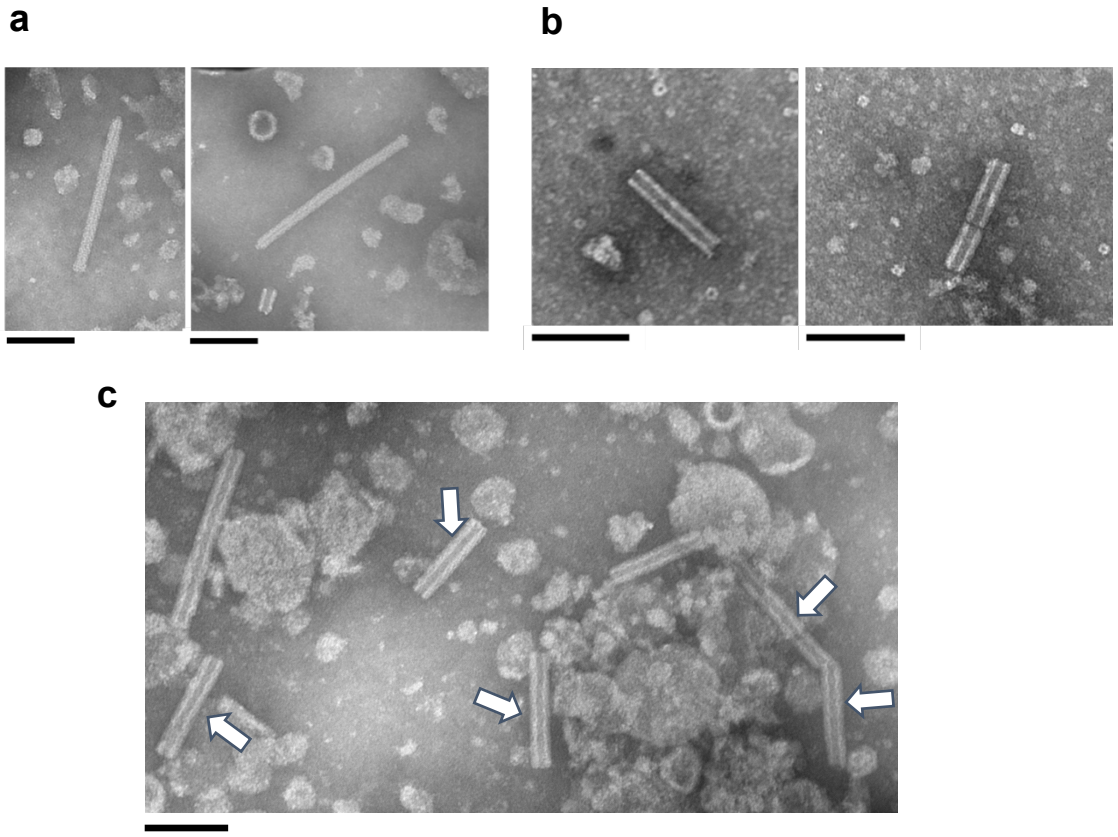
Supplementary Information

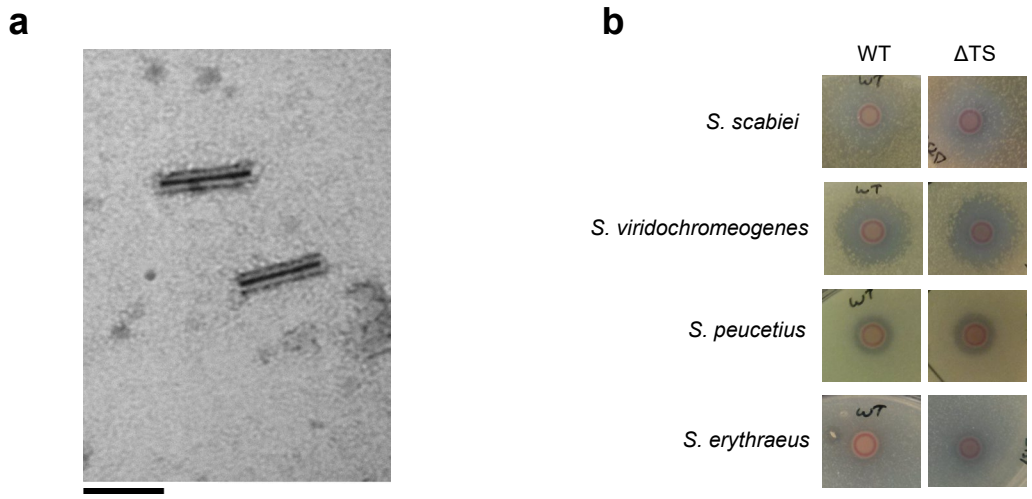
A contractile injection system is required for developmentally regulated cell death in *Streptomyces coelicolor*


a
24 hr
48 hr
96 hr
WT
ΔTS
WT
ΔTS
WT
ΔTS
His-TS (E.coli)
KDa
75
63
48
35
20
17
α-TS
b
E T S P KDa
Time post inoculation
42 hr
72 hr
- 63
α-TS
FLAG-ActR
- 25
α-FLAG
c
ΔTS
WT
ΔBP_1
ΔBP_2
T S P T S P T S P T S P
kDa
75
63
48
35
25
20
17
11
α-BW1
Non-specific, cytoplasmic protein
Sco4245 BW1
d
48 hr
68 hr
kDa
E S E S E S E S
α-FLAG
35 -
FLAG ActR (Cytoplasmic marker)
α-TS
48 -
Tail sheath
α-BW1
17 -
BW1
FLAG-ActR + + - - + + - -
ΔTS - - + + - - + +

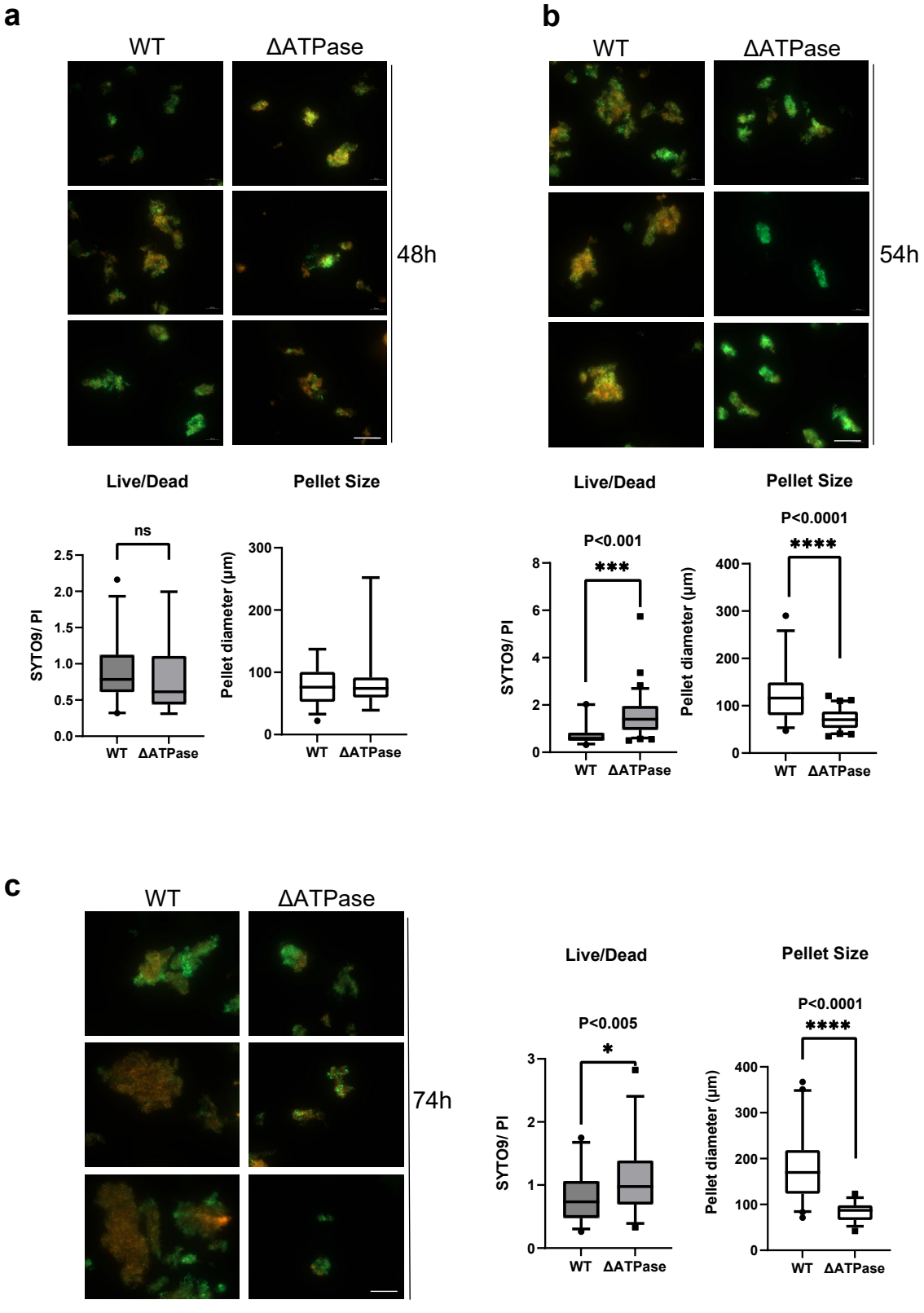
Supplementary Fig. 1| eCIS proteins are detected by specific polyclonal antibodies in lysates of WT *Sco*. a, *Sco* wild-type (WT) or a tail sheath knockout strain (ΔTS) were grown in liquid R2YE media to the indicated time points. Whole cell lysates were analyzed by Western blot using α-TS sera. Heterologously expressed and purified 6XHis-tagged-tail sheath (His-TS) was used as a positive control. **b,** WT *Sco* cells were grown in liquid YEME media, cells were collected at the indicated time points and an extracellular "E" sample was taken from the cell-free supernatant. Cells were lysed and an intracellular total lysate "T" sample was collected. The lysate was ultracentrifuged for 3 hr at 150,000 x g, the supernatant "S" and pellet "P" were collected. Samples were probed using α-TS antibodies. For the *Sco* strain expressing FLAG-tagged ActR from a plasmid, expression was induced with 30 µg/mL final concentration of thiostrepton, added 45 minutes before cells were harvested by centrifugation. **c,** *Sco* wild-type (WT) or a baseplate knockout (ΔBP) were grown and fractionated as in (b). Samples were probed by Western blot using α-BW1 antibodies. **d,** *Sco* expressing FLAG-ActR or a tail sheath knockout strain (ΔTS) were grown for 48 or 68 hr and separated as in (b). Extracellular "E" and lysate "S" samples were probed by Western blot using α-TS, α-BW1 or α-FLAG antibodies. Western blots show representative results from three independent fractionation experiments. Source unprocessed blots are provided within the Source Data file.



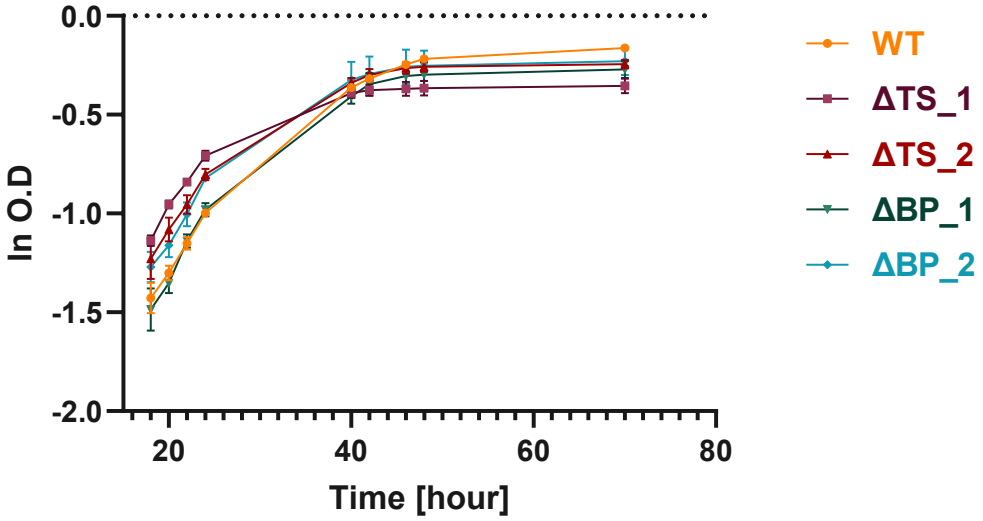
Supplementary Fig. 2| The *Sco* ATPase mutant produces both mature eCIS and empty sheath particles, detectable in the lysate and extracellular media. All images represent *Sco* ΔATPase grown in YEME liquid media for 48 hr (a), 54 hr (b) or 72 hr (c) post inoculation. **a,** Representative images of purified eCIS particles from cell lysate concentrated to 30X original culture after 48 hr of growth. The majority of particles at this time are in their extended conformation. **b,** The extracellular fraction after 54 hr of growth is shown. White arrows point to typical emptied contracted tail sheath particles. **c,** Purified lysate from cells grown for 72 hr. Emptied sheath particles are indicated with white arrows. Scale bar = 100 nm. The micrographs shown are representative of results obtained for three separate biological replicates.



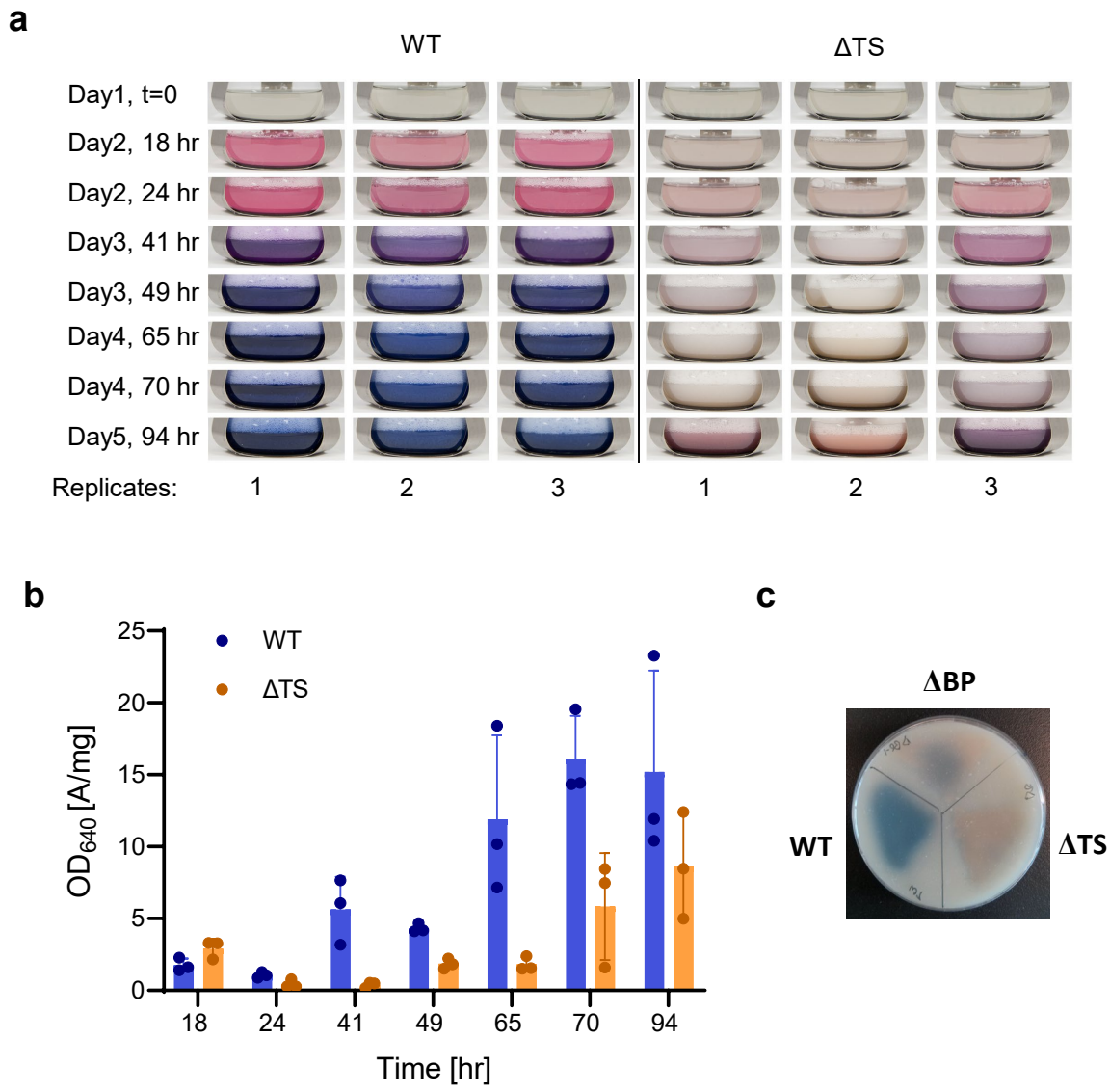
Supplementary Fig. 3| Growth inhibition of other *Streptomyces* species by WT and eCIS-deficient *Sco* strains. a, A representative TEM image of cell-free media collected from *Sco* M1152 strain grown for 72 hr. Empty contracted sheath particles, visually identical to ones produced by *Sco* WT M145 strain are observed. The presence of these particles is indicative of production of normal eCIS particles that can contract. No particles were detected in a M1152 strain harboring a tail sheath knockout mutation (M1152 ΔTS). Scale bar = 100 nm. **b,** Assays were performed by spotting on agar plates 10^5 SFU of *Sco* wild-type (WT) or tail sheath knockout (ΔTS) strains. Cells were grown for 3 days before being overlaid with species to be tested as indicated in each panel. Zones of clearing around the *Sco* colonies indicate lethality or growth inhibitory activity against the indicator lawn. The results shown for a tested species strain were obtained from the same agar plate. Images are representative examples of many replicates (n > 3).


a
WT
ΔATPase
48h
Live/Dead
Pellet Size
ns
SYTO9/ PI
Pellet diameter (µm)
WT
ΔATPase
b
WT
ΔATPase
54h
Live/Dead
Pellet Size
P<0.001
***
P<0.0001
****
SYTO9/ PI
Pellet diameter (µm)
WT
ΔATPase
c
WT
ΔATPase
74h
Live/Dead
Pellet Size
P<0.005
*
P<0.0001
****
SYTO9/ PI
Pellet diameter (µm)
WT
ΔATPase

Supplementary Fig. 4| The *Sco* ATPase deletion mutant strain exhibits reduced cell death during hyphal differentiation. a-c, *Sco* wild-type (WT) or an ATPase knockout (ΔATPase) strains were inoculated into YEME media at a final concentration of 10^7 SFU /mL. At 48 hr (**a**), 54 hr (**b**) or 74 hr (**c**) post-inoculation, samples were stained with equal proportions of propidium iodide and SYTO 9. Total (live and dead) cells are stained with SYTO 9 and are visualized by green fluorescence. Cells with damaged membranes are stained only with PI and are visualized by red fluorescence. The ratio of SYTO 9 to PI (Live/dead ratio) was calculated from total fluorescence intensity values per image. Diameters of individual hyphal pellets were manually measured from each collected image (for WT and ΔATPase, respectively; at 48 hr – n=37, 29, at 54 hr – n=37, 70, at 74 hr – n=28, 33). Results are shown as box plots. The center horizontal line denotes the median and boxes extend from the 25th to the 75th percentile of values. Whiskers denote values within the 5-95th percentile range, with individual data points shown for the values outside that range. Single factor ANOVA and Welch's t-test were performed to calculate statistical significance. Adjusted two-sided *P*-values are shown above the relevant graphs. In (b), *P*-value for live/dead ratios = 4.9E-04, *P*-value for pellet size =5.5E-10 . In (c), *P*-value for live/dead ratios = 0.0226 , *P*-value for pellet size = 9.5E-11. Scale bar = 100 μm.
Source data are provided as a Source Data file.



Supplementary Fig. 5| Growth curve for WT *Sco* and eCIS-deficient mutant strains. 10^8 SFU of *Sco* WT, tail sheath knockout (ΔTS) or baseplate knockout (ΔBP) were inoculated in R2YE liquid media and grown for 70 hours. Optical density (OD_{600}) was taken at the indicated time points. ΔTS_1, ΔTS_2 and ΔBP_1, ΔBP_2 represent two biological replicates of each mutant. The mean is shown with error bars representing standard error of the mean (n=3).



Supplementary Fig. 6| eCIS mutants exhibit decreased actinorhodin production in minimal media. a, Comparison of wild-type *Sco* (left) and ΔTS mutant (right) development in liquid media. Three experimental replicates of RG2 liquid media cultures were inoculated to an OD_{450} of 0.5 and grown for 94 hours. Images of each culture flask were taken at the indicated time points. **b,** For quantification of total actinorhodin, samples were taken at the same time points as in (a). KOH was added to a final concentration of 1 M to extract the actinorhodin and the sample was centrifuged. The optical density (OD_{640}) of the supernatant was measured and normalized to wet pellet weight. The mean is shown with error bars representing standard deviation of the mean (n=3). **c,** Equal amounts of *Sco* wild-type, ΔTS or ΔBP spores were streaked out on minimal Soy Mannitol (MS) agar plates and grown for 4 days. The agar plate shown is representative of multiple independent assays with 3 biological replicates (strains ΔTS 1-3 , ΔBP 1-3, each obtained by a separate conjugation event).

Promoter region *sco4248-49*

gatccacttggcgaccttcgccgtgtccggggtgaggggcgggtcagccggatgttgga
gtaggtgacgcgggtgggcagctgccagacgaagccgttgttgccgccctcctggcgctg
ttccacctccacctgggtcgacaggccctcgcacccgttgaagtagccgaggctctcgcc
gtcgatggcgaggctgaaccagatggaagagcccgggtccttacgggtcatcgggggtcg
acctttcttgtcggggagcggtggcggcgggcttggccctcggtgcggtccggccgtccg
gcggggccggtgcgggtccggtcagcgcgggtcgcgcagtctgccgatccgttcgcggtc
catacgcagttcggtgcggacgagacgggtgacgcggccgatgatccggtgcaccagttc
gtcgagctggaagtcggtgagttgacgcgggtcgaacccgccctcggggaccgccgtgta
cgcgggcgggggaccgtcgggggcggtgcgcccgcgcccgcctcgtacggcggcggggg
gtcgctgtacgggggcggcggttcgtccgggccgggccgggacggcgggggtccagccg
ggtcaggttgtccaccacggtggcggccgtggcggaggccagggcggcggtggtgtgtcc
ggcgcggcgggaacggcggaaggagaaccgctggaccggcggcatgtcgccggacacggc

Promoter region *sco4253-54*

ttcagcgggccggtcggggcgagaccgacgaaggccgccaccgacgtgcccactccctcg
atcgggcgcgagccgctggccacctcctcgacgtagacgccgggcgacaggtaggacggc
atgctctgctctcctcggggtacgagacaggacgcccctcagcctcacgcgcgcgttgcc
gatgccgaaacgtccccgggtgccggagcgggggcatgttccttgcccctggtcgtgccc
gttggggcagactcccgccgcatcccggcacttccccccgaagacccctggcggacgccc
ggcgtttctgattcaacgtcttgtgtcgactgtcagttcgccagaatgtcatcttgcgat
caagccccgtttcggggcgccccgtcccgccgcaccggggcgctgccccgcgaagagggg
acggtgacggcgtgtcaggaggggaccgtccgtgtcggaccgcttgaaccagacgcacag
gcaccgcccggccggggccgctccggctccggcccgcccgcaggcacggcagtcgccgcc
gctggcctcgttgcaggccgcggcggggaaccgggccgtggcctcggcgatccagcgcgc
ggccggtaccgcacagctctccaaggccccggcaccggaacgggcgacgacctcgtcgac
gccggacgacctgcagatcacaccggtgacggagcccgagcagcaggcggccgtggacga

Supplementary Fig. 7| *Sco* eCIS promoter regions. The DNA sequence of the eCIS promoter regions of *Sco* is shown as diagrammed in Fig. 1b. The two divergent promoter regions are indicated by red blocks[1]. The translational start codons (for *sco4248* and *sco4249* on the top and *sco4253* and *sco4254* on the bottom) are underlined in red. Primers used to clone the promoter regions into the *luxCDABE* reporter plasmid are highlighted in yellow and indicated by arrows above the sequence.

Supplementary Tables

eCIS protein	WT	ΔBP	WT	ΔBP
	Intracellular		Extracellular	
TR (DUF4255)				
Tail sheath				
Tail tube				
BH1				
BH2				
BW1				
BW2				
BW3				
Sco4242				
Sco4251				
Sco4256				
ATPase				

Supplementary Table 1| Summary of LC-MS/MS analysis of purified *Sco* eCIS samples. eCIS-associated proteins identified through LC MS/MS analysis of purified *Sco* eCIS particles (Intracellular) or in concentrated purified extracellular media (Extracellular). Hits detected in three separate purifications are highlighted in dark grey. Hits identified in fewer than three experiments are highlighted in light grey. Only proteins with E-values better than 10^{-5} were considered a hit. Source data have been deposited in the MassIVE database under accession code; MSV000091288 [doi:10.25345/C5CC0V388].

Gene name	Protein accession	Function	log(e)	log(I)	% Coverage	Total spectra	Molecular mass
Sco4260	NP_733632	Terminator / DUF4255	-46.6	6.91	42	5	24.7
Sco4253	NP_628427.1	Tail sheath	-251.7	8.22	77	77	56.9
Sco4252	NP_628426.1	Tail tube	-150	7.57	83	40	16.5
Sco4246	NP_628420.1	BH2/spike	-65	6.62	24	7	68
Sco4244	NP_628418.1	BW2/gpJ	-190.1	7.52	52	25	70.8
Sco4242	NP_628416.1		-46.7	6.98	26	8	48.5
Sco4256	NP_628430.1	Hydrolytic	-59.4	6.95	27	8	51,3

Supplementary Table 2| Detailed results of LC-MS/MS analysis of WT eCIS samples.
Wild-type *Sco* were purified as described in Methods. The samples were reduced, trypsin digested and C18 purified before LC-MS and data processing. Values shown are from one representative run.
Log(e): the base-10 log of the expectation that any protein assignment was made at random.
Log(I): the base-10 log of the sum of the fragment ion intensities in the tandem mass spectra used to make this assignment.
% Coverage: the amino acid coverage of the protein in this assignment divided by the coverage corrected for peptide sequences that are unlikely to be observed using normal proteomics methods.
Total spectra: the total number of tandem mass spectra that can be assigned to this protein.

Supplementary Table 3: Strains and plasmids used in this study.

Strains	**Description**	**Reference or source**
***Escherichia coli* strains**		
DH5α		Stratagene
ET12567/pUZ8002	Conjugation donor strain, Kan^R, Cml^R	[2]
BW25113/pIJ790	λ Red recombination strain, Cml^R	[3]
BL21(DE3)	Protein expression	Stratagene
Stellar HST08	High transformation efficiency competent cells	Clontech
***Streptomyces coelicolor* strains**		
M145	Wild-type *S. coelicolor* A3(2)	
WT::pf*lux*-EV	Promoterless *luxCDABE* operon	[4]
WT::pf*lux*-hrdBp	*luxCDABE* operon driven by the hrbB sigma factor promoter	[4]
WT::pf*lux*-redDp	*luxCDABE* operon driven by the RedD promoter	[4]
WT::pf*lux*-eCISp1	*luxCDABE* operon driven by the eCIS promoter P1	This study
WT::pf*lux*-eCISp2	*luxCDABE* operon driven by the eCIS promoter P2	This study
WT::pf*lux*-eCISp3	*luxCDABE* operon driven by the eCIS promoter P3	This study

Strains	**Description**	**Reference or source**
WT::pf*lux*-eCISp4	*luxCDABE* operon driven by the eCIS promoter P4	This study
M1152	Derivative of M1146, Δ*act*, Δ*red*, Δ*cpk,* Δ*cda*, *rpoB* [C1298T]	[5]
M145 ΔTS (*Δsco4253::apra*)	eCIS tail sheath gene *sco4253* replaced with apramycin resistance cassette	This study
M145 ΔBP (Δ*sco4243*Δ*sco4244*Δ*sco4245::apra*)	eCIS baseplate genes *sco4243-45* replaced with apramycin resistance cassette	This study
M145 ΔATPase (Δ*sco4259::apra*)	eCIS ATPase gene *sco4259* replaced with apramycin resistance cassette	This study
M1152 ΔTS (Δ*sco4253::apra*)		This study
M145::pIJ6902-actR-FLAG	M145 conjugated with pIJ6902 plasmid encoding inducible FLAG-tagged ActR (Cytoplasmic control)	[6]
M145::pIJ6902-S4646-FLAG	M145 conjugated with pIJ6902 plasmid encoding inducible FLAG-tagged SecE (Membrane control)	[6]
Plasmids		
pFlux	Reporter plasmid, Promoterless luxCDABE operon, ApraR	[4]

Strains	**Description**	**Reference or source**
pFlux-eCISp1	eCIS promoter region P1 (Sco4248-4249 reverse)	This study
pFlux-eCISp2	eCIS promoter region P2 (Sco4248-4249 forward)	This study
pFlux-eCISp3	eCIS promoter region P3 (Sco42453-4254 reverse)	This study
pFlux-eCISp4	eCIS promoter region P4 (Sco42453-4254 forward)	This study
pIJ7331	pBluescript KS (+), aac(3)IV, oriT (RK2)	[7]
p15TV-L	T7 expression vector, derivative of pET15b, AmpR, (GenBank ID: EF456736.1)	Novagen
p15-SC4245	6XHis BW1 expression plasmid	This study
p15-SC4253	6XHis tail sheath expression plasmid	This study
Cosmids		
StD8a		[8]
StD8aΔsco4253::apra		This study
StD8aΔsco4243-45::apra		This study
StD8aΔsco4259::apra		This study

Supplementary Table 4: Oligonucleotides used in this study.

Oligonucleotide	**Sequence (5'– 3')**
Lux reporter cloning	
SCO_eCISp1p2GEN_F	GCTGCCAGACGAAGCCGTTGTTGCCGC
SCO_eCISp1p2GEN_R	CACCGTGGTGGACAACCTGACCCGGCT
SCO_eCISp1p2EcoRV_F	GGGATATCGGATCCCAGACGAAGCCGTTGTTGCCGC
SCO_eCISp1p2EcoRV_R	CCGATATCTGGTGGACAACCTGACCCGGCT
SCO_eCISp3p4GEN_F	GCGAGACCGACGAAGGCCGCCACCGA
SCO_eCISp3p4GEN_R	GGGCCTTGGAGAGCTGTGCGGTACCGGC
SCO_eCISp3p4EcoRV_F	GGGATATCGGATCCGACCGACGAAGGCCGCCACCGA
SCO_eCISp3p4EcoRV_R	CCGATATCTTGGAGAGCTGTGCGGTACCGGC
Gene disruption	
SCO4253_Disrp_F	GGGGCGTCCTGTCTCGTACCCCGAGGAGAGCAGAGCATG ATTCCGGGGATCCGTCGACC
SCO4253_Disrp_R	TAGAGGATGTGGGACAAGGGCGGGGGCGCGATCCGCCTA TGTAGGCTGGAGCTGCTTC
SCO_BP_Disrp_F	GACGAAGCGTTTCCGAGGAAGGCAGGTTGCTCCCTGATGA TTCCGGGGATCCGTCGACC
SCO_BP_Disrp_R	CCGCGGGGGTTCCGCACTCGGCGCAGTTCTGCGTGGTCAT GTAGGCTGGAGCTGCTTC
SCO4259_Disrp_F	CGTGACGGACGGCCGCACGAAACGACAGTGACGACCGTG ATTCCGGGGATCCGTCGACC
SCO4259_Disrp_R	AAGAAGGGCTGACGGCCGGAGGGGGCCCGGCGGCCGTCA TGTAGGCTGGAGCTGCTTC

Oligonucleotide	**Sequence (5'– 3')**
Gene replacement verification	
StD8aΔTS_aac(3)IV _F	GAAGTGCCGGGATGCG
StD8aΔTS_aac(3)IV _R	GCAGGTTGGAGACCGAGTTG
StD8aΔBP_aac(3)IV _F	CGTCAACGTGCAGATCAACTCC
StD8aΔBP_aac(3)IV _R	CGAAGGCGTCGAAGCCGGGGCG
StD8aΔATP_aac(3)IV _F	CGACCCGCCGATGTCCGAGG
StD8aΔATP_aac(3)IV _R	CGGCGCCCTCTTCTCCAAG
Recombinant protein cloning	
p15TV-L SCgpW_F	TTGTATTTCCAGGGCATGGCCGAACAGTTCGTC
p15TV-L SCgpW_R	CAAGCTTCGTCATCATCAGCGGTCGCTTTCG
p15TV-L SC_Vdmn_F	TTGTATTTCCAGGGCATGGTCGCCAACGCCATC
p15TV-L SC_Vdmn_R	CAAGCTTCGTCATCA GTCGCTCTTGGTCGGC
p15TV-L SCTS_F	TTGTATTTCCAGGGCATGCCGTCCTACCTGTCG
p15TV-L SCTS_R	CAAGCTTCGTCATCACTACTCGTCCAGTTCGCCG

Supplementary References

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