

SUPPORTING INFORMATION

Defining the regulon of genes controlled by σ^E , a key regulator of the cell envelope stress response in *Streptomyces coelicolor*

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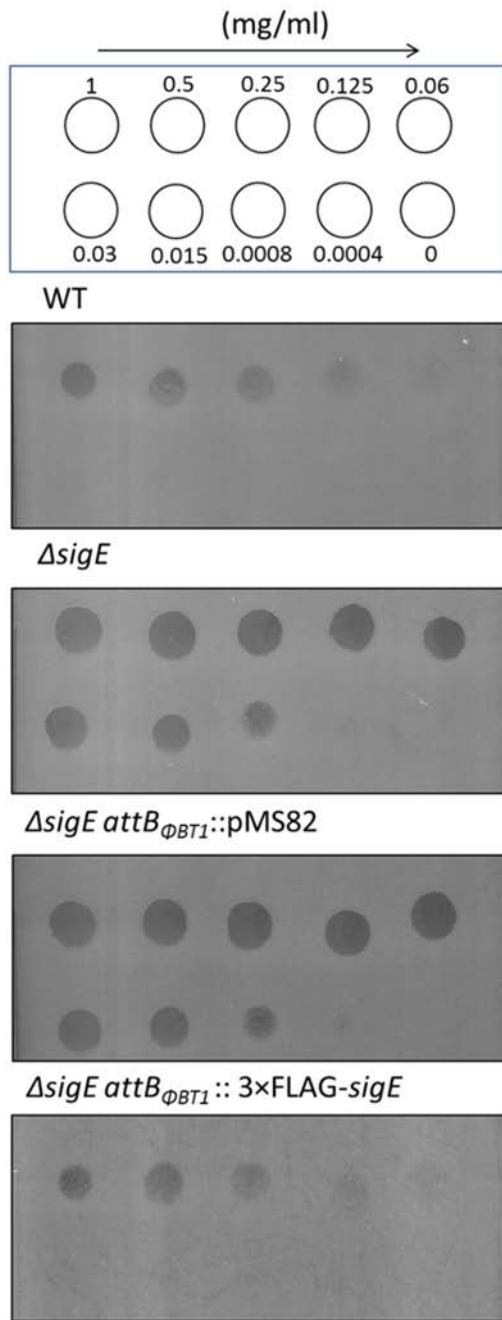
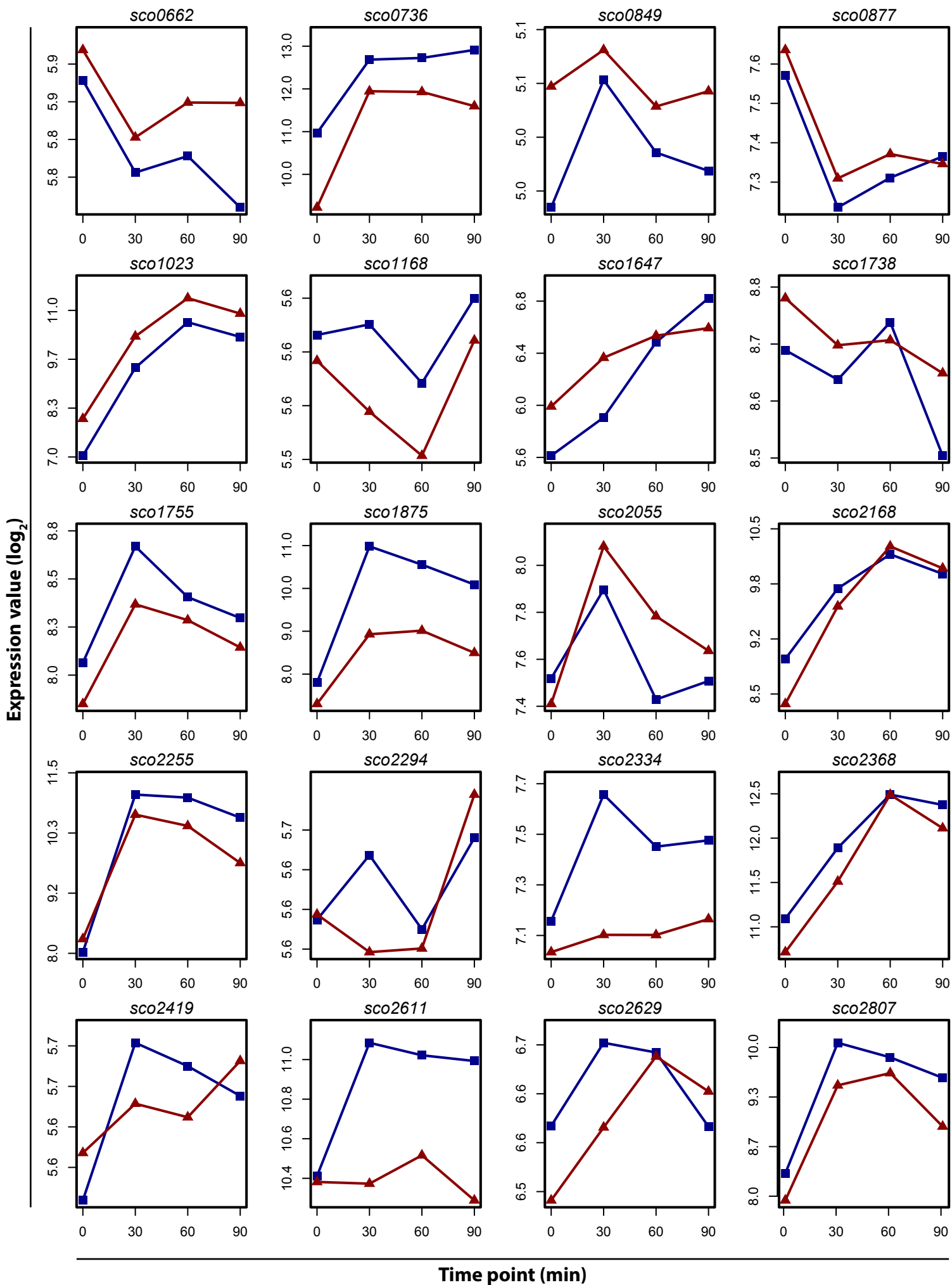
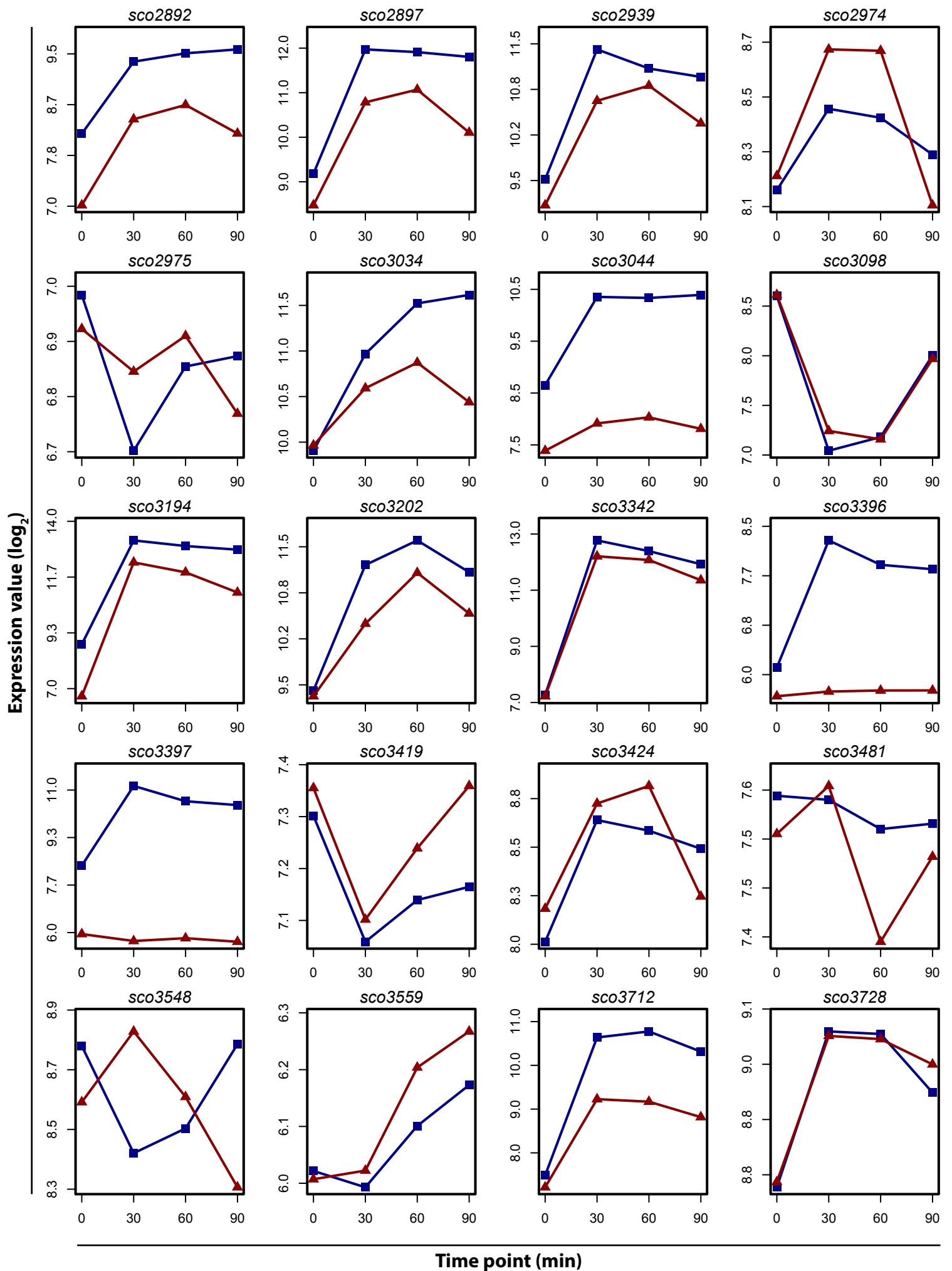


Fig. S1. Lysozyme sensitivity of wild-type *S. coelicolor* M600 (WT), the *sigE* null mutant J2130 ($\Delta sigE$), J2130 harbouring the empty pMS82 vector ($\Delta sigE attB_{\phi BT1}::pMS82$), and J2130 expressing the N-terminal, triple FLAG-tagged σ^E ($\Delta sigE attB_{\phi BT1}::3\times FLAG-sigE$). 5 μ l lysozyme in a 2-fold dilution series was spotted onto DNA agar immediately after plating 2×10^6 spores of each strain to create a confluent lawn. Images were taken after 2 days of growth at 30°C.

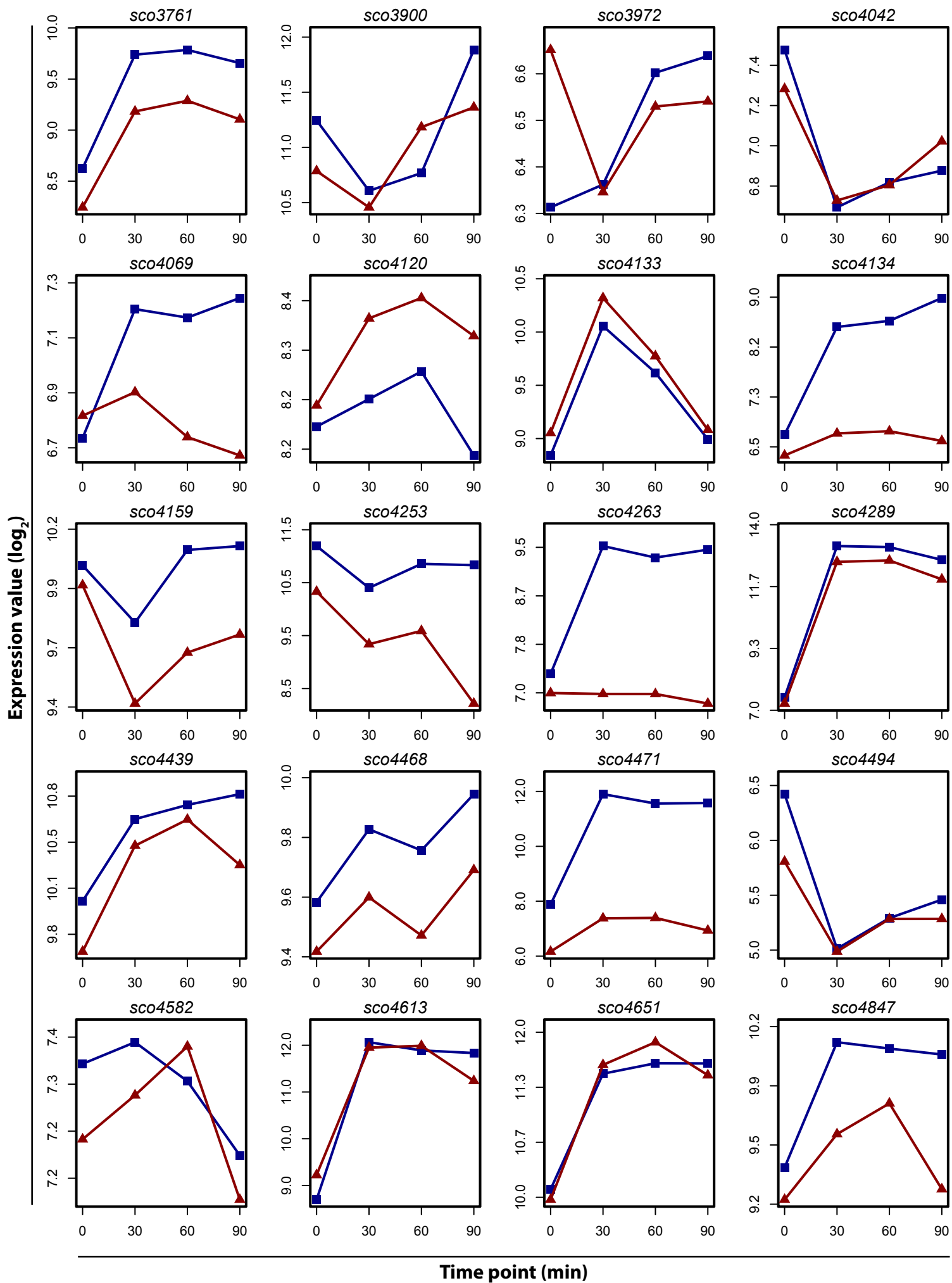
■ WT ▲ $\Delta sigE$



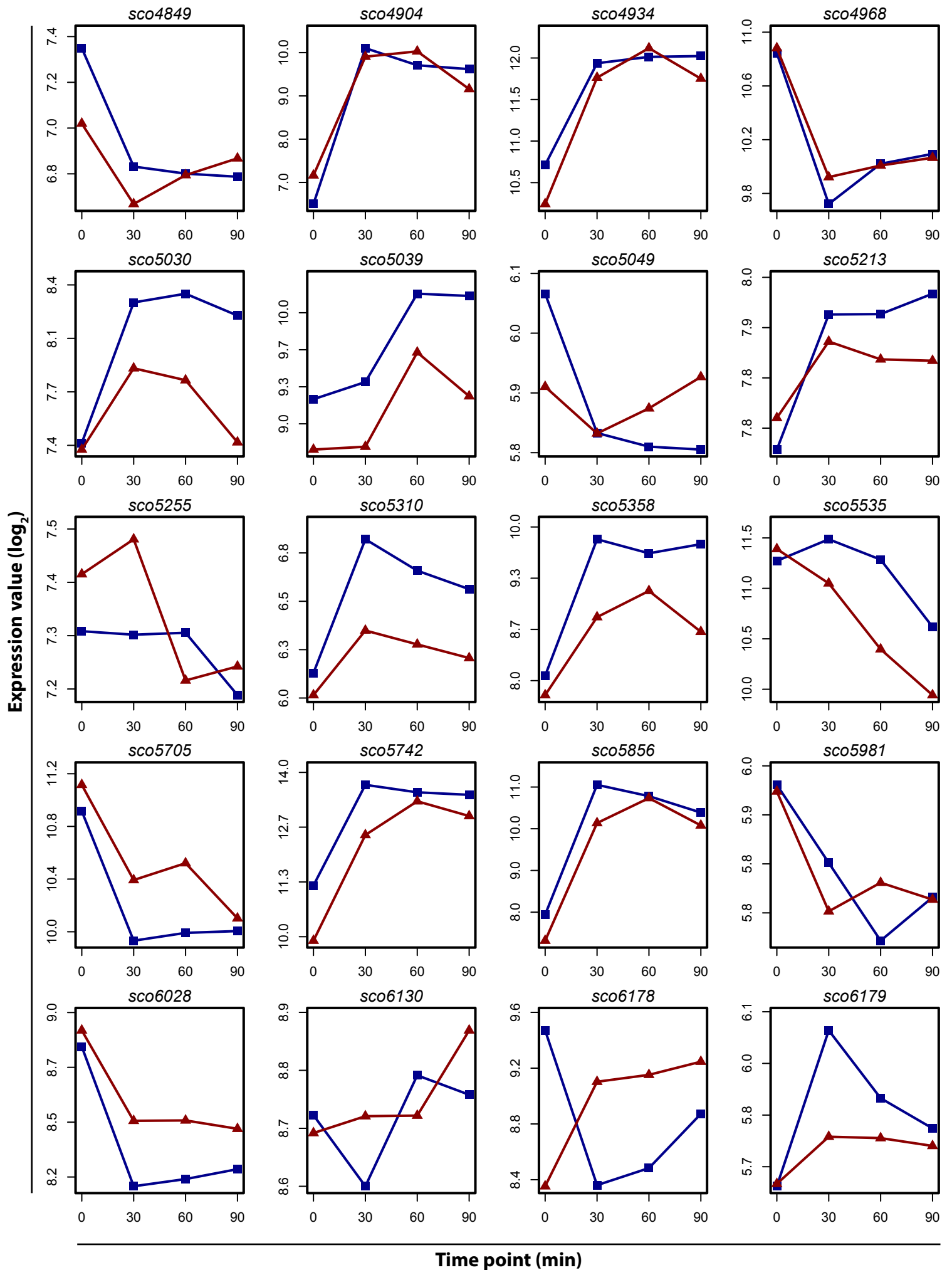
■ WT ▲ $\Delta sigE$



■ WT ▲ $\Delta sigE$



■ WT ▲ $\Delta sigE$



■ WT ▲ $\Delta sigE$

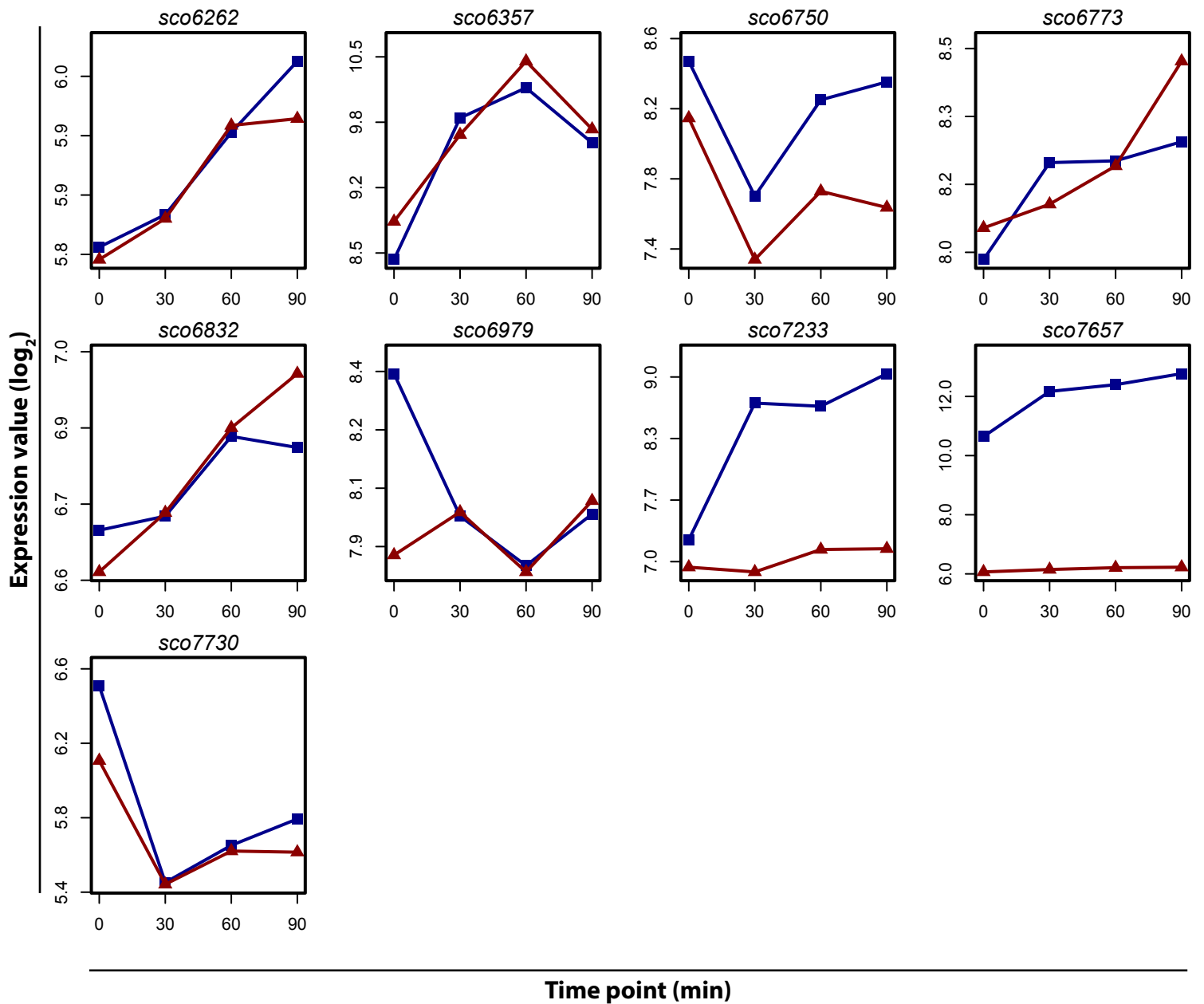


Fig. S2. Time-resolved microarray transcriptional profiling data for all the σ^E target genes listed in Table 1. Color-coding of the microarray data is as follows: *S. coelicolor* M600 (WT, blue squares), *sigE* null mutant J2130 ($\Delta sigE$, red triangles). In each panel the x-axis indicates the time in minutes (0, 30, 60 or 90) after the addition of 10 μ g/ml vancomycin, and the y-axis indicates the per gene normalized transcript abundance (log₂). Note that two of the σ^E target genes listed in Table 1, the tRNA *scot11* and *sco6379*, were not represented on the Affymetrix Gene Chip and so no transcriptional profiling data are available for these two genes.

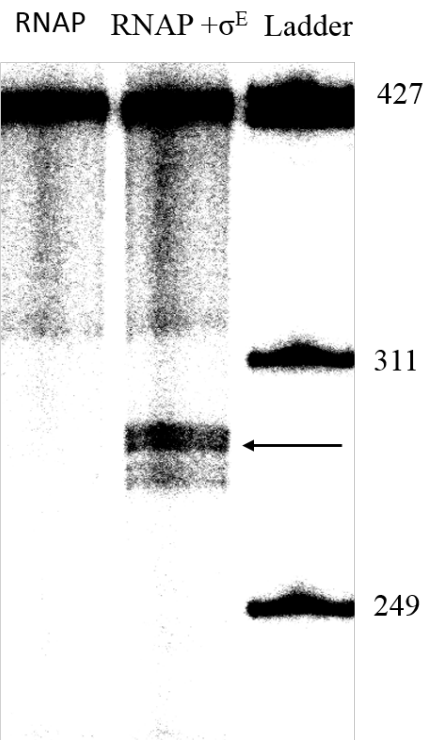
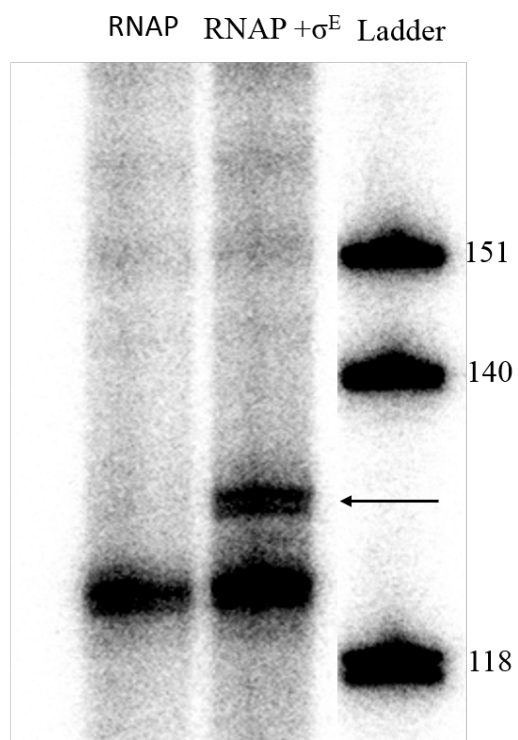
A*Sco3396***B***Sco4471*

Fig. S3. *in vitro* run-off transcription assays on the σ^E target genes (A) *sco3396* and (B) *sco4471*. In each case a transcript of the expected size (indicated by the arrow) is only detected in the presence of RNAP and σ^E . The sizes of the standards (a heat-denatured ^{32}P -labelled *Hpa*II digest of pBR322) are indicated in nt.

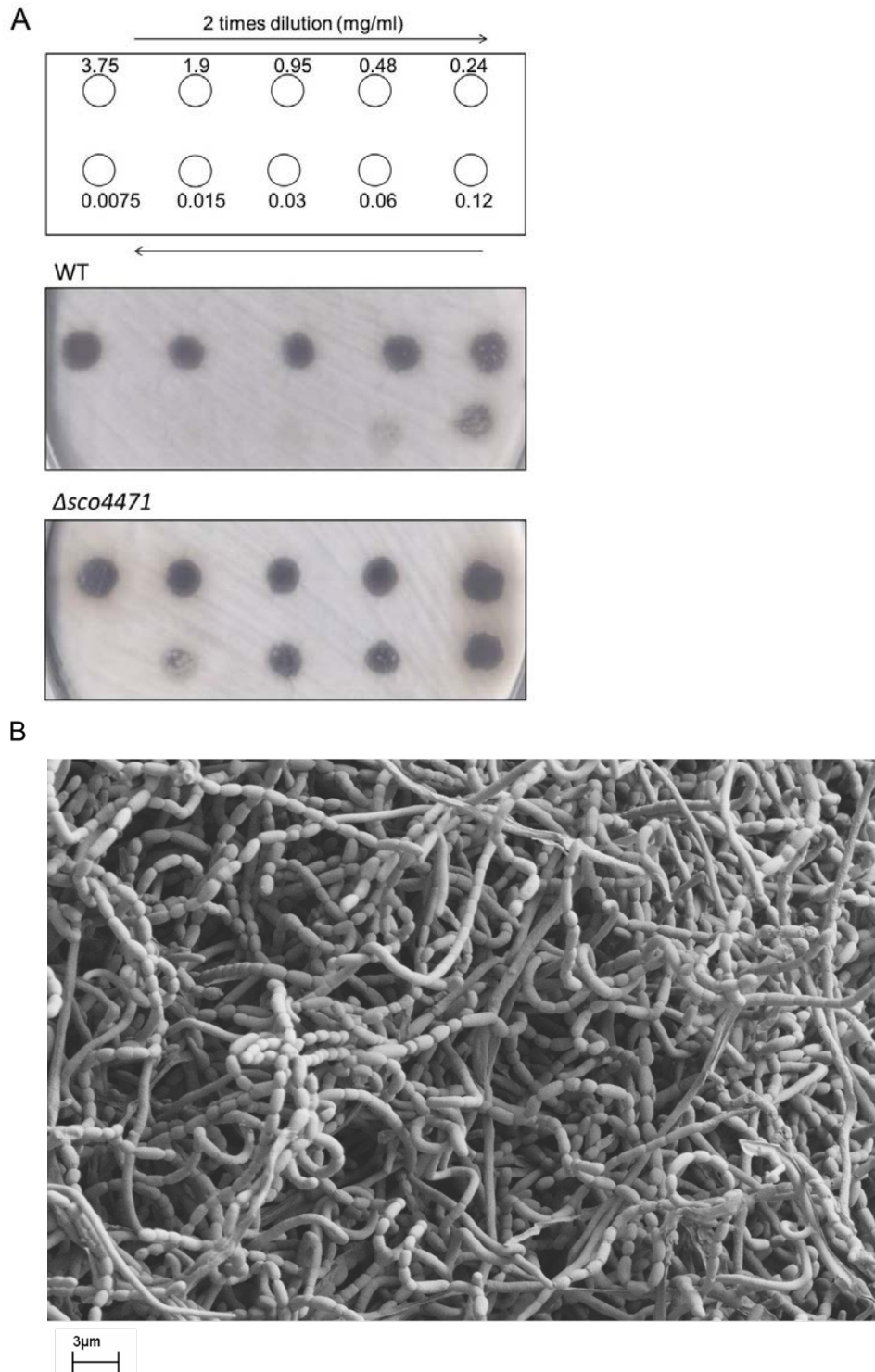


Fig. S4. (A) Lysozyme sensitivity of $\Delta sco4471::apr$ mutant and the wild type *S. coelicolor* M600 (WT). A 2-fold dilution series of lysozyme was spotted onto DNA agar immediately after plating 2×10^6 spores of each strain to create a confluent lawn. Images were taken after 2 days of growth at 30°C. (B) Scanning electron microscopy image of the $\Delta sco4471::apr$ mutant showing minor abnormalities in spore size and shape. Imaged after 5 days of growth on SFM medium.

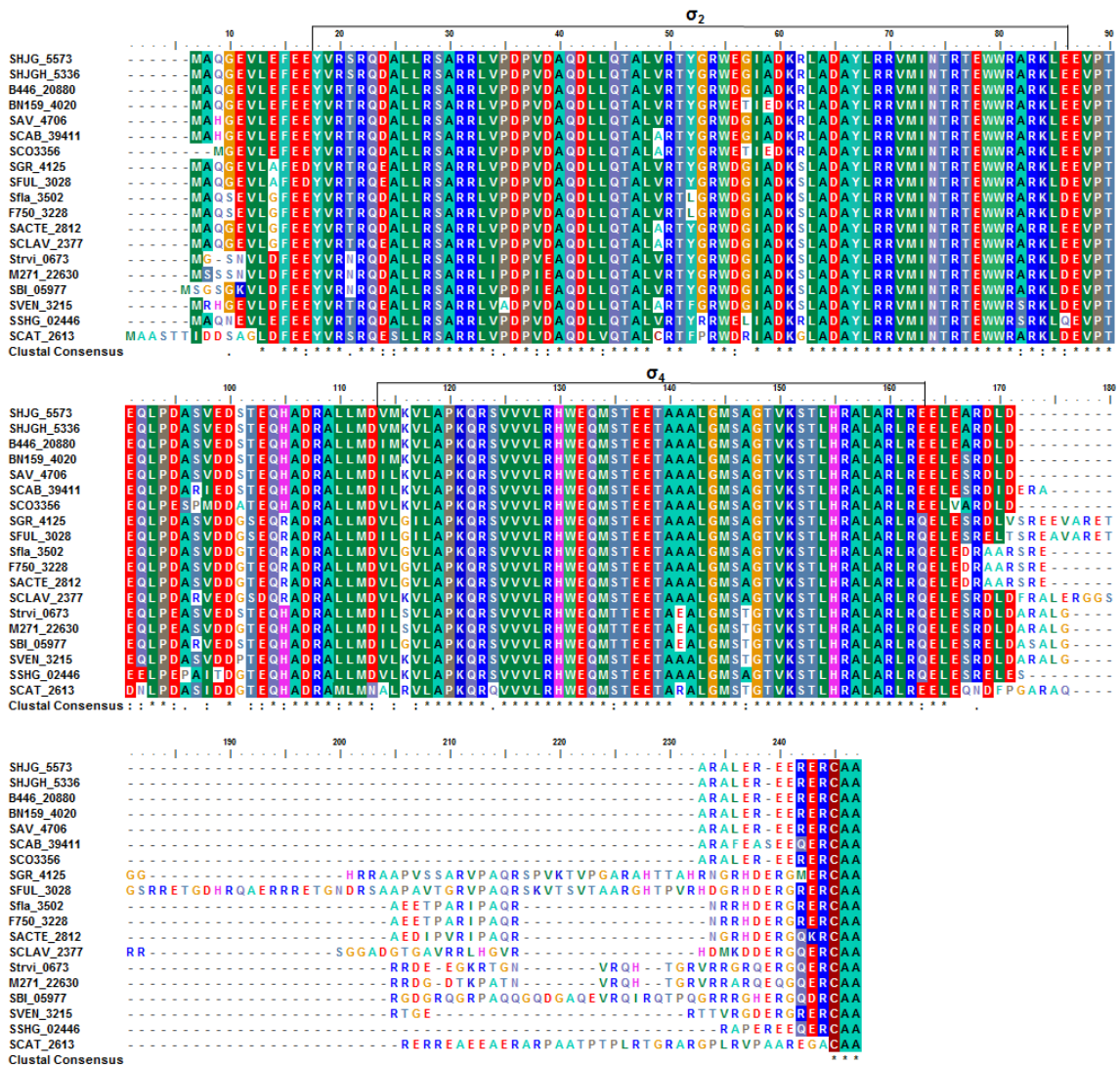


Fig. S5. Conservation of σ^E orthologs across 19 *Streptomyces* genomes. Multiple alignment of the amino acid sequences of the σ^E orthologs from 19 *Streptomyces* species (as listed in Table S2) were carried out by EMBL-EBI ClustalW2 online program (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). SCO3356 is *S. coelicolor* σ^E . The σ_2 and σ_4 domains were identified using the SMART database (<http://smart.embl-heidelberg.de/>) and are indicated in the figure.

Table S2. The 19 *Streptomyces* genomes used to analyze conservation of *S. coelicolor* σ^E target promoters

Accession¹	Species	Abbreviation
CM000913	<i>Streptomyces clavuligerus</i> ATCC 27064	<i>Scla</i>
NC_003155	<i>Streptomyces avermitilis</i> MA-4680 = NBRC 14893	<i>Save</i>
NC_003888	<i>Streptomyces coelicolor</i> A3(2)	<i>Scoe</i>
NC_010572	<i>Streptomyces griseus</i> subsp. <i>griseus</i> NBRC 13350	<i>Sgri</i>
NC_013929	<i>Streptomyces scabiei</i> 87.22	<i>Ssca</i>
NC_015953	<i>Streptomyces</i> sp. SirexAA-E	<i>SspS</i>
NC_015957	<i>Streptomyces violaceusniger</i> Tu 4113	<i>Svio</i>
NC_016111	<i>Streptomyces cattleya</i> NRRL 8057 = DSM 46488	<i>Scat</i>
NC_016114	<i>Streptomyces pratensis</i> ATCC 33331	<i>Spra</i>
NC_016582	<i>Streptomyces bingchengensis</i> BCW-1	<i>Sbin</i>
NC_017765	<i>Streptomyces hygrosopicus</i> subsp. <i>jinggangensis</i> 5008	<i>Shyg_1</i>
NC_018750	<i>Streptomyces venezuelae</i> ATCC 10712	<i>Sven</i>
NC_020504	<i>Streptomyces davawensis</i> JCM 4913	<i>Sdav</i>
NC_020895	<i>Streptomyces hygrosopicus</i> subsp. <i>jinggangensis</i> TL01	<i>Shyg_2</i>
NC_020990	<i>Streptomyces albus</i> J1074	<i>Salb</i>
NC_021055	<i>Streptomyces</i> sp. PAMC26508	<i>SspP</i>
NC_021177	<i>Streptomyces fulvissimus</i> DSM 40593	<i>Sful</i>
NC_021985	<i>Streptomyces collinus</i> Tu 365	<i>Scol</i>
NC_022785	<i>Streptomyces rapamycinicus</i> NRRL 5491	<i>Srap</i>

¹The Genbank accession number (<http://www.ncbi.nlm.nih.gov/genbank/>) for the 19 *Streptomyces* genomes.

Table S3. Bacterial strains, plasmids and oligonucleotides.

	Relevant genotype/comments	Source/reference
<i>S. coelicolor</i>		
M600	SCP1 ⁻ SCP2 ⁻	(1)
J2130	$\Delta sigE$ M600	(2)
J3354	$\Delta SCO4471::apr$ M600	This work
<i>E. coli</i>		
DH5 α	F ⁺ <i>supE44</i> $\Delta lacU169$ ($\Phi 80lacZ$ $\Delta M15$) <i>hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	(3)
ET12567	F ⁺ <i>dam13::Tn9 dcm6 hsdM hsdR recF143::Tn10 galK2 galT22 ara-14 lacY1 xyl-5 leuB6 thi-1 tonA31 rpsL hisG4 tsx-78 mtl-1 glnV44</i>	(4)
ET12567 /pUZ8002	ET12567 containing helper plasmid pUZ8002	(2)
BW25113	<i>lacI^q rrnB_{T14} $\Delta lacZ_{WJ16}$ hsdR514 $\Delta araBAD_{AH33}$ $\Delta rhaBAD_{LD78}$</i>	(5)
BL21 (DE3) /pLysS	F ⁻ <i>ompT gal dcm lon hsdS_B (r_B⁻m_B⁻)</i> λ (DE3 [<i>lacI lacUV5-T7 gene 1 ind1 sam7 nin5</i>]) pLysS (Cam ^R)	(6)
Plasmid		
pMS82	Plasmid integrating at the Φ BT1 attachment site (Hyg ^R) of <i>S. coelicolor</i>	(7)
pIJ773	Plasmid template used for the amplification of the <i>apr oriT</i> cassette in 'Redirect' PCR-targeting	(8)
pIJ790	Modified λ RED recombination plasmid [<i>oriR101</i>] [<i>repA101(ts)</i>] <i>araBp-gam-be-exo</i>	(8)
pMS82-3FLAG- <i>sigE</i>	pMS82 harboring 3 \times FLAG- <i>sigE</i> driven by its native promoter	This work
Primer		
	Primer sequences (5'-3')	
SCO3396S1FW	CGATGTCCAGCAGGCCGAC	
SCO3396S1RV	GGTGGCGAGCAGCAGACCG	
SCO3397S1FW	CCCAGAACGGCCCCGCACACTCCG	
SCO3397S1RV	GACGAGCACGCAGGCGCGGCCGAC	
SCO2897S1FW	CTGCCACGGAATTTACGGGTG	
SCO2897S1RV	TAGACGTTGGACTGCTTGGTG	
SCO4134S1FW	CCACGTAGCCGCAGGCAATC	
SCO4134S1RV	AGGTGAGTGCGGTGCGCGTTC	
SCO3194S1FW	TGGTGACGCGGGCGATCGTGC	
SCO3194S1RV	GCCGCAGGCGGTGAGGGTGAG	
SCO4471S1FW	CTCCGTCGGCTACACCGAGCCC	
SCO4471S1RV	GAACCGGTGCCGCAGCCCCGCGAG	
SCO5030S1FW	GGCAAGTACGCCTCCGCGGGC	
SCO5030S1RV	TTCTCGCGGCGCTGCTCCAAC	
SCO7233S1FW	TCCAGCGACGACTGCCCGTTC	
SCO7233S1RV	TACGGATGCCGGGCCGCTG	
SCO2334S1FW	CGATGGAACCTTCCTCGCAAAG	
SCO2334S1RV	CGAGCAGCGGCACGCGATG	
SCO2611S1FW	GTCTGATCCTTCTTCGGGA	
SCO2611S1RV	TTGGCGGTCCCGAGGTCGAC	
SCO5358S1FW	CACCTACGCCTCCACCTG	
SCO5358S1RV	CGCGACGAGCAGGGCCTTG	
SCO4934S1FW	AGTGGGACCTCGACGAGGATG	
SCO4934S1RV	GGAACATGCCGAGAGCACCAG	

SCO3712S1FW	ACCGTCGTCTACACGGCCG
SCO3712S1RV	ACCACCGTGCCGCGCCATG
SCO4263S1FW	TCCGGAGCGGACACCGCCAC
SCO4263S1RV	GCAGGACGAGGCGGCCGCTG
SCO3044S1FW	TCGCGCGTTTTGTCGAGTGCGCGAAGTGACCT
SCO3044S1RV	GCTGGTAGTAGAGGTATCCGG
SCO7657S1FW	CAACACCTTGCACGTGGTCACC
SCO7657S1RV	GAGTCCGGCGGTCACGGCCATG
SCO4847S1FW	CGTCGTATCCCCTCGGTTG
SCO4847S1RV	ACGGACTCGTCGACGGGCTC
SCO4471KOFW	CCAGTGACCACCAAGGACGCGACGAACGAG
	GTTGCGATGATTCCGGGGATCCGTCGACC
SCO4471KORV	GGAAACCCGCATTTACAGGCACCGTTTCGCGG
	TCGCTTCATGTAGGCTGGAGCTGCTTC
P1 _{3NFLAG-SigE}	AAAAAAGCTTACCAGGTGTGGCAGGAGC
P2 _{3NFLAG-SigE}	TCGATGTTCGTGGTTCCTTGTAGTCGCCGTCGTG
	GTCCTTGTAGTCCATGTCAACCGCCTTCCG
P3 _{3NFLAG-SigE}	CGACTACAAGGACCACGACATCGACTACAAG
	GACGATGACGACAAG
	GGCGAGGTGCTCGAGTTC
P4 _{3NFLAG-SigE}	AAAAGGTACCCACCGCCGTACTIONCGCC

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