

## Supplemental data

### **Molecular control of polyene macrolide biosynthesis: direct binding of the regulator PimM to eight promoters of pimaricin genes. Identification of binding boxes.**

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**Table S1: Primers used in RT-PCR experiments.**

**Table S2: Primers used for 5' RACE experiments.**

**Table S3: Primers and probes used for the EMSA assays.**

### **Fig. S1: Identification of binding sites.**

DNaseI footprints of the GST-PimM<sup>DBD</sup> protein bound to the promoter regions of *pimK* (A,B), *pimI* (C,D), *pimA* (E,F), *pimE* (G,H). In each panel, the upper electropherogram (blue line) is the control reaction. The protected nucleotide sequence is boxed; hyper sensitive sites (arrows) are also indicated. Sequencing reactions are not included. Coordinates are from the transcriptional start point.

**Table S1: Primers used in RT-PCR experiments**

<b>Primer</b>	<b>Sequence (5' to 3')</b>	<b>Intergenic region</b>
S3S4f	GCCGTCCTTCAACAACCTGCCAG	<i>pimS3-pimS4</i>
S3S4r	GCCCGACGCCCGAACCAG	
S2S3f	CTTCCTGGCTGACGGCGTTGC	<i>pimS2-pimS3</i>
S2S3r	CTGCTGAAGCTCGCCGCCG	
IS2f	GCCGCCGCCTCCAGCTCGC	<i>pimI-pimS2</i>
IS2r	GACGACCCCAAGACCACGCTG	
JIf	CGTGCGGAAAGCAGACGAGC	<i>pimJ-pimI</i>
JIr	CRACTTCCCCGAACCTGATGCG	
CGf	GCGGGACGTGCTGTGGATGTAC	<i>pimC-pimG</i>
CGr	GCCATAACCGTTTGCCGTCG	
GFf	GGGACGCTCATCAAGGCGGG	<i>pimG-pimF</i>
GFr	CGGACGGGCACAGGGCG	
FS0f	GTCACCCTCCTCACCAGCCC	<i>pimF-pimS0</i>
FS0r	CGATACCCGACTCCTGCAACCC	

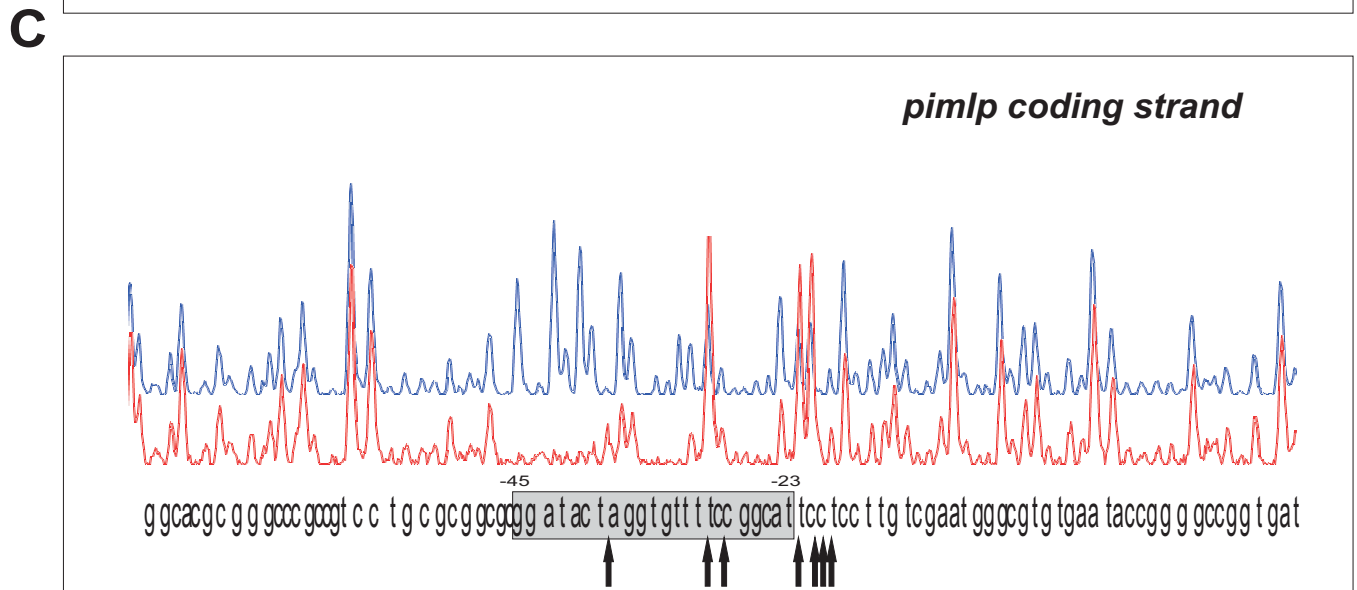
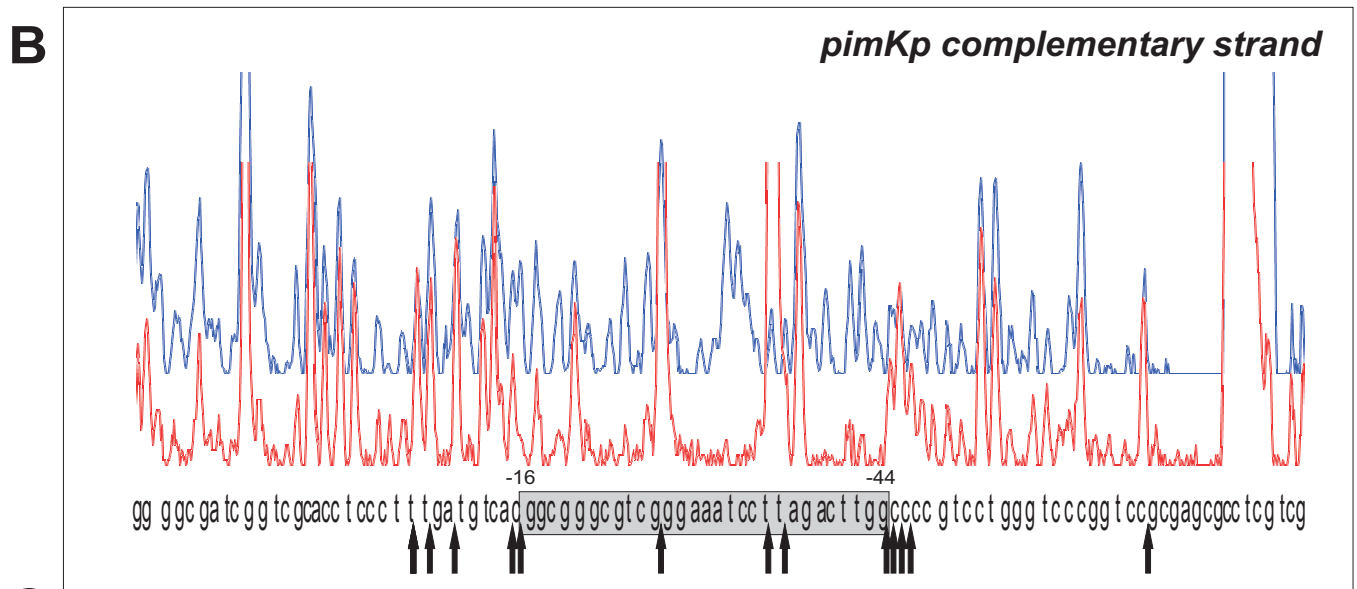
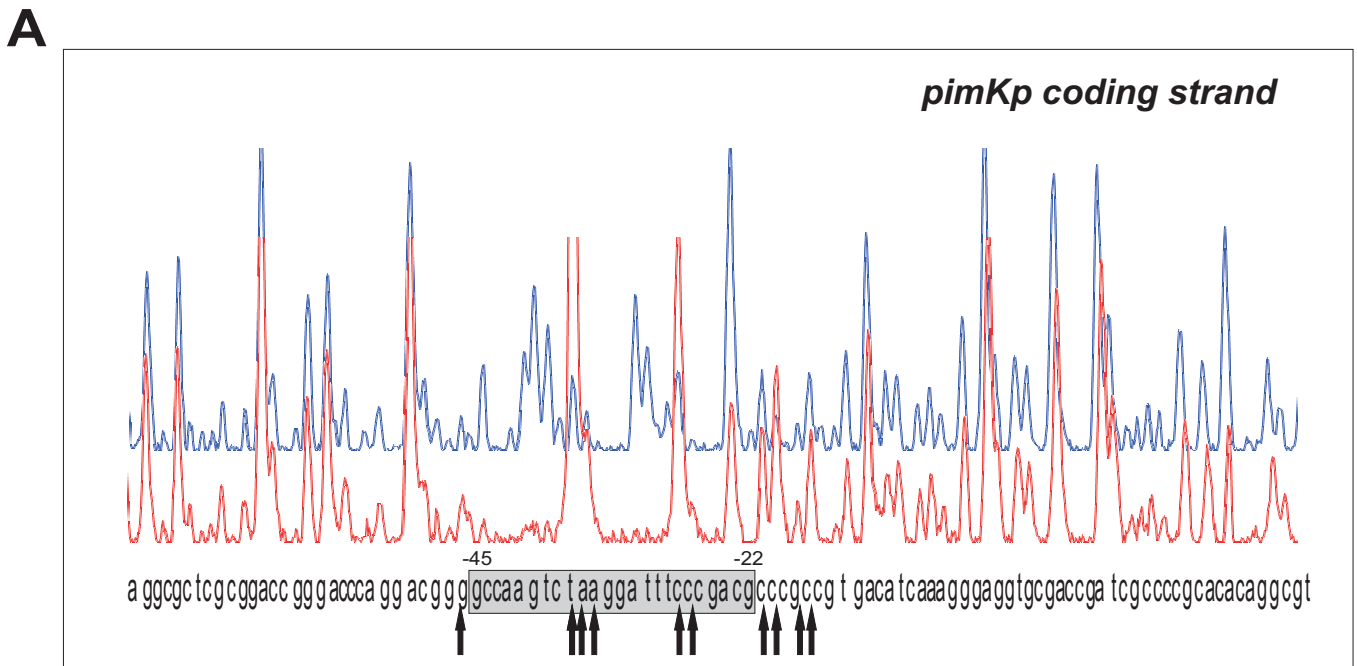
**Table S2: Primers used in 5' RACE experiments**

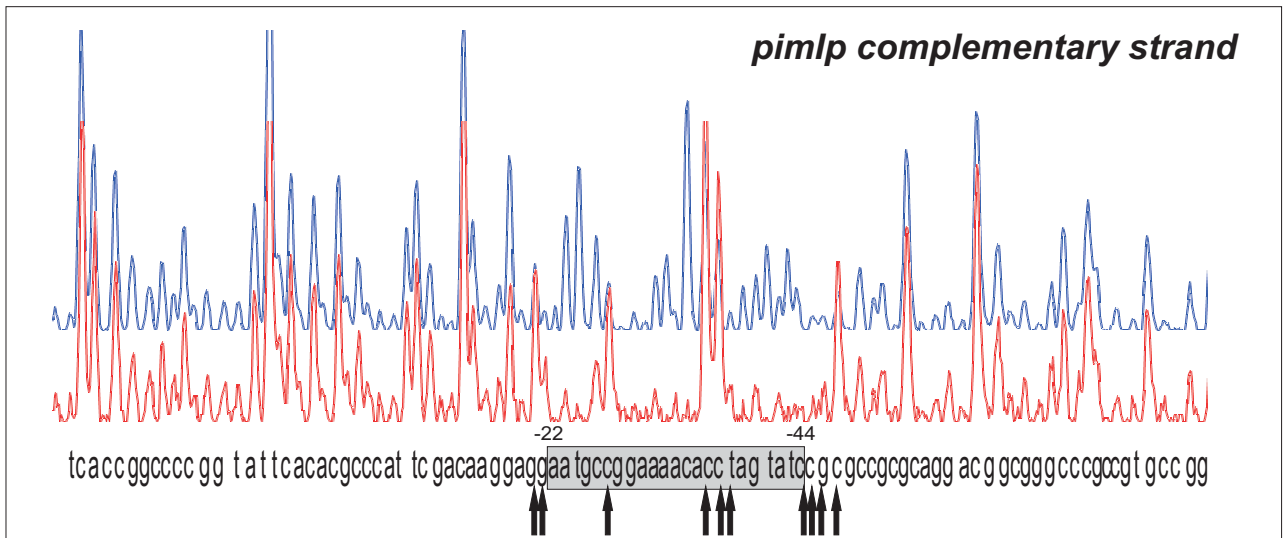
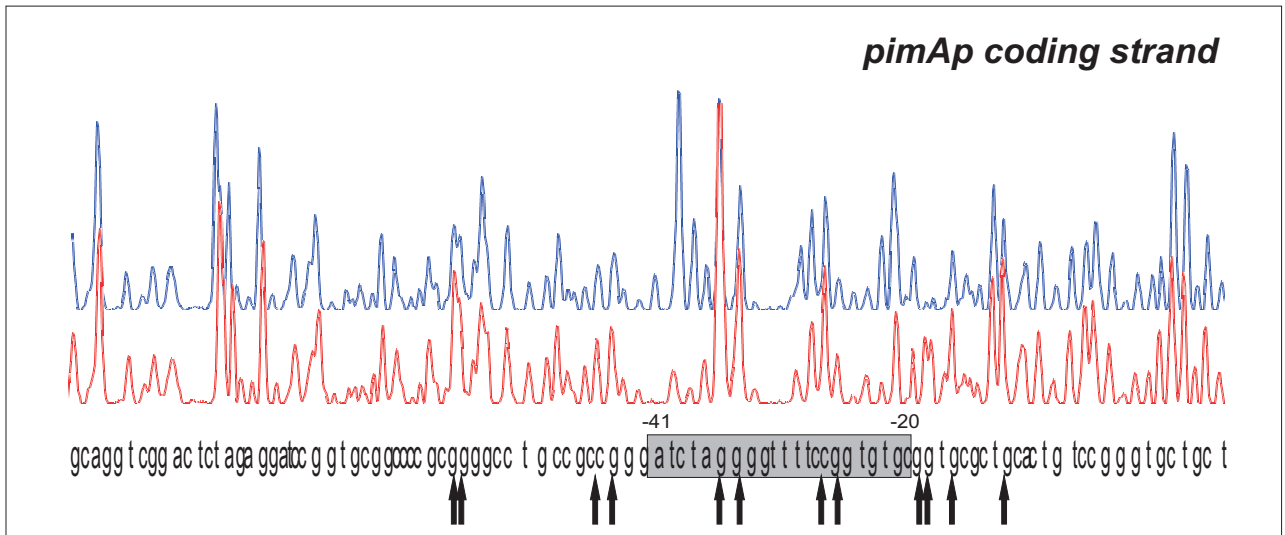
<b>Primer</b>	<b>Sequence (5' to 3')</b>	<b>Transcription initiation site determination</b>
K-1	GCAGGAACGGCACGCTCAGTACG	<i>pimK</i>
K-2	GGCTTGGAGACGGCGGAACTTC	
K-3	CCAGCGAGGCGAACTCCACAG	
S2-1	CGAGATGACGCTGGTGGTGGTGC	<i>pimS2</i>
S2-2	CGAAGGCTTCCCAGGAGGACTCC	
S2-3	GAAGAAGGAGGCGTCGAACTCGG	
I-1	GGGATCGCAGCGGTAGGTCTCC	<i>pimI</i>
I-2	CGGAGAGCAGCTTCAGTTCGG	
I-3	CCACCTCGAAGGCGACCATC	
J-1	GGTAGTTGCGGGTGATGAAGTGCC	<i>pimJ</i>
J-2	CGACGAGGACGCCTGGTAGAACC	
J-3	CTCGTCGGGCTGCACCTTGTCG	
A-1	GCCACCAGGAGCAGGGAGAGC	<i>pimA</i>
A-2	GACACGACGACGCCGAACGC	
A-3	GGGTGATGTAGCCGCTGTTCG	
E-1	GAGCCGCCGCCGACACC	<i>pimE</i>
E-2	CCAGCCACAGGAACGAGCCG	
E-3	GTCGTCGGCGGGCTTGTTCC	
S1-1	GGAGGCGATGCTGATGTGATTGC	<i>pimS1</i>
S1-2	CGCTCGACGGCTTCCCATGC	
S1-3	CGGGTCGGAGTGGTAGAGCG	
D-1	GCGTCCAGCAGGGTGTCCG	<i>pimD</i>
D-2	CACCTTCGGCTGCATTTCCAGAACG	
D-3	GAGCAGGCGGCGGGTCTCG	

**Table S3: Primers and probes used for the EMSA assays.**

Putative promoter region	Primer	Sequence (5' to 3') <sup>a</sup>	Probe size (bp)
<i>pimT</i>	xTf	<u>GGATCC</u> GTGCCCGTCACCTCC	143
	xTr	GAATTCGTGTGTCCAGGTGACCGTC	
<i>pimM-R</i>	xMf	CAAGGATCCGAGCTGAAGGTGCTGGAC	380
	xMr	GTTGAATTCGTCCCCGTGCCTCTCG	
<i>pimK</i>	pKf	CCTGGATCCCTGATGCACACCTCGCTG	462
	pKr	CAACAAGCTTCACCCGCCAGCACGAG	
<i>pimS4</i>	pS4f	GGCTATCGGATCCTGCTTGGCGG	384
	pS4r	CCTCCTGGCAGAATTCGAACGGCTC	
<i>pimS3</i>	pS3f	GCGGTCAGCGGATCCACAGGTCC	362
	pS3r	CGTGCTCGAATTCCTGCTGAAGCTCG	
<i>pimS2</i>	pS2f	AAATGGATCCTCGCCACCCGCTGCC	443
	pS2r	CCGAAGCTTCGACGACCCCAAGACCACGC	
<i>pimI</i>	pIf	GCGAGCGGGAATTCGGGGACCTGCTG	280
	pIr	ACACAGGATCCGCCGTGGTCGTCGTCATGG	
<i>pimJ</i>	pJf	GACTAAGCTTCACAGCTGGACTGGTCCATCAAGTCGCC	465
	pJr	CCGCACCGGATCCGGCTGCGCCAG	
<i>pimA</i>	pAf	CTGGGCGCAGCCGGATCCGGTGCGG	104
	pAr	GATGGATTCGCAGAAGACATAGCAGCACCG	
<i>pimE</i>	pEf	GAACGGATCCCGCACGGAAGTCCTCATCCAACG	432
	pEr	GAGAAAGCTTGCGGCATGAGGGGCGGAGGTGATC	
<i>pimC</i>	pCf	CATGGATCCGCTACCACAACCTCTACGTCACGGACGGCG	377
	pCr	GACTAAGCTTCCGTGCGCGACGCCGTTGTAGTCCG	
<i>pimS1-D</i>	pS1Df	GACTAAGCTTCAGCGGGCTCAGTTTCAGCATTTTGGGC	440
	pS1Dr	GAGGGATCCGCTCTCGCTGCTTCGCCTCGGTCTCGTCC	
<i>pimH</i>	xHf	CGCTGCCGCTGTTGCCG	492
	xHr	GATCCCGGACGAATTCCTGGTCAC	
<i>amphI</i>	AmIf	GACTGGAAGGAATTCGTCGTCGTCGACCC	427
	AmIr	GCCGTCGCAAGCTTGAGGTAATCC	
<i>amphJ</i>	AmJf	GCCGCCTGGATCCCGTTCGTGGACC	513
	AmJr	CAACTACGTTCTCAGCGGGGGCG	
<i>amphK</i>	AmKf	GGTGTGCGGATCCCGTCCACGC	451
	AmKr	GGTCTGGTGGGGATCCGCCGTGACC	

<sup>a</sup> Introduced restriction sites are underlined.



**D****E****F**