Activating natural product synthesis using CRISPR interference and activation systems in Streptomyces.

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Supplementary table 1. List of all plasmids used in this study. Abbreviations are as follows: R9 = R9 ribosome binding site, ori = origin of replication, specR = spectinomycin resistance gene, apmR = apramycin resistance gene, oriT = origin of transfer, sgRNA = single guide RNA, ds origin = double-stranded origin, bp = base pairs, NT = non-template strand, T = template strand, αNTD = N-terminal domain of the α subunit of RNAP. Promoters: KasO*p, ermE*p, gapdh(EL), rpsL(XC), 57, SP43, SP30, SP20, SP10, SP1. Terminators: T7, λt0, Fd. Origins of replication: pMB1, pUC.

Plasmid ID	Plasmid features	Name	Figure(s)
pJEC532	KasO*p - mCherry - T7 - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	mCherry reporter	1b, 1c, 1d, S1
pJEC533	gapdh(EL) - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	gapdh(EL)-mCherry	S1
pJEC710	Fd terminator - gapdh(EL) - lacZ - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	no CRISPR	1b, 1c, 1d, 2b, 2c, 3d, 3e, S1, S2, S3
pJEC711	rpsL(XC) - dCas9 - Fd terminator - gapdh(EL) - mCherry sgRNA (+11bp) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	rpsL(XC)-dCas9/gapdh(EL)-sgRNA	1b
pJEC712	rpsL(XC) - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+11bp) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	rpsL(XC)-dCas9/SP43-sgRNA	1b, 1c
pJEC713	SP1 - RiboJ - R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+11bp) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP1-dCas9/SP43-sgRNA	1c
pJEC714	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+11bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+11 NT)	1c, 1d, S2
pJEC715	SP43 - RiboJ -R9 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	SP43-mCherry	S1
pJEC716	SP30 - RiboJ -R9 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	SP30-mCherry	S1
pJEC717	ermE*p - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	ermE*p-mCherry	S1
pJEC718	SP20 - RiboJ -R9 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	SP20-mCherry	S1
pJEC719	SP10 - RiboJ -R9 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	SP10-mCherry	2b, 2c, S1

pJEC720	rpsL(XC) - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	rpsL(XC)-mCherry	S1
pJEC721	57 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	57-mCherry	S1
pJEC722	SP1 - RiboJ -R9 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	SP1-mCherry	S1
pJEC723	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - non-coding genomic region sgRNA #1 - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	CRISPRi with sgRNA binding to a genomic region	1d,S2
pJEC724	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - non-coding genomic region sgRNA #2 - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	CRISPRi with sgRNA binding to a genomic region	S2
pJEC725	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - no-match sgRNA #2 - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA off-target w/o binding site	S2
pJEC726	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - lacZ - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA scaffold (i.e. no sgRNA control)	S2
pJEC727	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+123bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+123 NT)	1d
pJEC728	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+230bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+230 NT)	1d
pJEC729	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+531bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+531 NT)	1d
pJEC730	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+623bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+623 NT)	1d
pJEC731	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+9bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+9 T)	1d

pJEC732	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+112bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+112 T)	1d
pJEC733	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+223bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+223 T)	1d
pJEC734	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+540bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+540 T)	1d
pJEC735	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - mCherry sgRNA (+627bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9/SP43-sgRNA (+627 T)	1d
pJEC736	PAM region - SP10 - RiboJ -R9 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	CRISPRa reporter #1	2b, 2c, S3
pJEC737	SP30 - RiboJ -R9 - dCas9 - XTEN - αNTD - Fd terminator - SP43 - non-coding genomic region sgRNA #1 - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-αNTD/off-target	2b
pJEC738	SP30 - RiboJ -R9 - dCas9 - XTEN - αNTD - Fd terminator - SP43 - CRISPRa sgRNA (82bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-αNTD/sgRNA -82 T	2b
pJEC739	SP30 - RiboJ -R9 - dCas9 - XTEN - αNTD - Fd terminator - SP43 - CRISPRa sgRNA (83bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-αNTD/sgRNA -83 NT	2b, 2c, S3
pJEC740	SP30 - RiboJ -R9 - dCas9 - XTEN - ω - Fd terminator - SP43 - non-coding genomic region sgRNA #1 - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-ω/off-target	2b
pJEC741	SP30 - RiboJ -R9 - dCas9 - XTEN - ω - Fd terminator - SP43 - CRISPRa sgRNA (83bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-ω/sgRNA -83 NT	2b
pJEC742	SP30 - RiboJ -R9 - dCas9 - XTEN - ω - Fd terminator - SP43 - CRISPRa sgRNA (82bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-ω/sgRNA -82 T	2b

pJEC743	SP30 - RiboJ -R9 - dCas9 - XTEN - RbpA - Fd terminator - SP43 - non-coding genomic region sgRNA #1 - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-RbpA/off-target	2b
pJEC744	SP30 - RiboJ -R9 - dCas9 - XTEN - RbpA - Fd terminator - SP43 - CRISPRa sgRNA (83bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-RbpA/sgRNA - 83 NT	2b
pJEC745	SP30 - RiboJ -R9 - dCas9 - XTEN - RbpA - Fd terminator - SP43 - CRISPRa sgRNA (82bp T) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-RbpA/sgRNA - 82 T	2b
pJEC746	PAM region + 5bp - SP10 - RiboJ -R9 - mCherry - T7 terminator - pMB1 ori - SpecR - RP4 oriT - ΦC31 attP site - ΦC31 integrase	CRISPRa reporter #2 (PAMs shifted by 5bp)	2c, S3
pJEC747	SP30 - RiboJ -R9 - dCas9 - XTEN - αNTD - Fd terminator - SP43 - CRISPRa sgRNA (73bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-αNTD/sgRNA - 73 NT	2c, S3
pJEC748	SP30 - RiboJ -R9 - dCas9 - XTEN - αNTD - Fd terminator - SP43 - CRISPRa sgRNA (93bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	SP30-dCas9-αNTD/sgRNA - 93 NT	2c, S3
pJEC749	SP30 - RiboJ -R9 - dCas9 - Fd terminator - SP43 - CRISPRi jadR2 sgRNA (+44bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	jadR2 CRISPRi	3d
pJEC750	SP30 - RiboJ -R9 - dCas9 - XTEN - αNTD - Fd terminator - SP43 - CRISPRa jadJ sgRNA (-73bp NT) - sgRNA scaffold - λt0 terminator - pUC ori - ApmR - pSG5 replicase - pSG5 ds origin - RP4 oriT	jadJ CRISPRa	3e

Supplementary table 2. Example DNA plasmid sequences.

Name and features	DNA sequence
CRISPRi reporter (Promoter-RBS-mCherry-Terminator-E.coli ori-SpecR-oriT-attP site-phiC31 integrase)	CGAGACACCCGGGAAGCCTGATCTACGTCTGTCGAGAAGTTTCTGATCG ATGACACTCGTTCGTTCACACGTTGCAGCAGAGTACTTGTTCACATTCGA ACGGTCTCTGCTTTGACAACATGCTGTGCGGTGTTGTAAAGTCGTGGCC AGGAGAATACGACAGCGTGCAGGACTGGGGGAGTGCGCATATGGTCTC CAAGGGCGAGGAGGACAACATGGCCATCATCAAGGAGTTCATGCGCTTC AAGGTCCACATGGAGGGCTCCGTCAACGGGCACGAGTTCGAGATCGAG GGCGAGGGGGAGGGCCGGCCGTACGAGGGCACCCAGACCGCCAAGCT GAAGGTGACCAAGGGCGGCCCCCTCCCGTTCGCCTGGGACATCCTCTC CCCCCAGTTCATGTACGGCTCGAAGGCCTACGTCAAGCACCCGGCCGA CATCCCGGACTACCTGAAGCTCTCGTTCCCGGAGGGGTTCAAGTGGGA GCGGGTCATGAACTTCGAGGACGGCGGCGTCGTCACCGTCACCCAGGA CAGCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTCAAGCTGCGGGG CACGAACTTCCCGAGCGACGGCCCCGTGATGCAGAAGAAGACGATGGG CTGGGAAGCGTCCTCGGAGCGCATGTACCCGGAGGACGGCGCCCTCAA GGGCGAGATCAAGCAGCGCCTGAAGCTGAAGGACGGCGGCCACTACGA CGCCGAAGTCAAGACGACGTACAAGGCCAAGAAGCCGGTGCAGCTCCC GGACGCCTACAACGTGAACATCAAGCTCGACATCACCTCGCACAACGAG GACTACACGATCGTGGAGCAGTACGAGCGCGCCGAGGGCCGGCACTCG ACCGGCGGCATGGACGAGCTGTACAAGTGAGCCGTTCGCGCCGCCCCG GCTCGCATCGTCCCCGACCCGTCAACCTCATCCGCAAGGAGTCTCTAGA GGATCCGCGGCCGCGCGCGATATCGAATTCCTCGAGTAACTAGCATAAC CCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGTTAAGCCGAAC AGGAAGCACAGCTCCTACTGAACATGTGAGCAAAAGGCCAGCAAAAGGC CAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGC CCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAA ACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCT CGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCC TTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGT ATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGA ACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTT GAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT GAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCT GCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTG ATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAG CAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTT TTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATT TTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTGGTTCAT GTGCAGCTCCATCAGCAAAAGGGGATGATAAGTTTATCACCACCGACTAT TTGCAACAGTGCCGTTGATCGTGCTATGATCGACTGATGTCATCAGCGG TGGAGTGCAATGTCATGCGCTCACGCAACTGGTCCAGAACCTTGACCGA ACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTCATGGCTTGTTATGAC TGTTTTTTTGGGGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGC GTTACGCCGTGGGTCGATGTTTGATGTTATGGAGCAGCAACGATGTTAC GCAGCAGGGCAGTCGCCCTAAAACAAAGTTAAACATC ATGAGGGAAGCGGTGATCGCCGAAGTATCGACTCAACTATCAGAGGTAG TTGGCGTCATCGAGCGCCATCTCGAACCGACGTTGCTGGCCGTACATTT

	GTACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGATATTGAT TTGCTGGTTACGGTGACCGTAAGGCTTGATGAAACAACGCGGCGAGCTT TGATCAACGACCTTTTGGAAACTTCGGCTTCCCCTGGAGAGAGCGAGAT TCTCCGCGCTGTAGAAGTCACCATTGTTGTGCACGACGACATCATTCCGT GGCGTTATCCAGCTAAGCGCGAACTGCAATTTGGAGAATGGCAGCGCAA TGACATTCTTGCAGGTATCTTCGAGCCAGCCACGATCGACATTGATCTGG CTATCTTGCTGACAAAAGCAAGAGAACATAGCGTTGCCTTGGTAGGTCCA GCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGATCTATTTGAGG CGCTAAATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGG CGATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCA GTAACCGGCAAAATCGCGCCGAAGGATGTCGCTGCCGACTGGGCAATG GAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGACAG GCTTATCTTGGACAAGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGT TGGAAGAATTTGTCCACTACGTGAAAGGCGAGATCACCAAGGTAGTCGG CAAATAATTGTCTTTCTTCAGCTCGCTGATGATATGCCTTCCTGGTTGGC TTGGTTTCATCAGCCATCCGCTTGCCCTCATCTGTTACGCCGGCGGTAG CCGGCCAGCCTCGCAGAGCAGGATTCCCGTTGAGCACCGCCAGGTGCG AATAAGGGACAGTGAAGAAGGAACACCCGCTCGCGGGTGGGCCTACTT CACCTATCCTGCCCGGCTGACGCCGTTGGATACACCAAGGAAAGTCTAC ACGAACCCTTTGGCAAAATCCTGTATATCGTGCGAAAAAGGATGGATATA CCGAAAAAATCGCTATAATGACCCCGAAGCAGGGTTATGCAGCGGAAAA GATCCGTCGACCTGCAGGCATGCAAGCTCTAGCGATTCCAGACGTCCCG AAGGCGTGGCGCGGCTTCCCCGTGCCGGAGCAATCGCCCTGGGTGGGT TACACGACGCCCCTCTATGGCCCGTACTGACGGACACACCGAAGCCCC GGCGGCAACCCTCAGCGGATGCCCCGGGGCTTCACGTTTTCCCAGGTC AGAAGCGGTTTTCGGGAGTAGTGCCCCAACTGGGGTAACCTTTGAGTTC TCTCAGTTGGGGGCGTAGGGTCGCCGACATGACACAAGGGGTTGTGAC CGGGGTGGACACGTACGCGGGTGCTTACGACCGTCAGTCGCGCGAGCG CGAGAATTCGAGCGCAGCAAGCCCAGCGACACAGCGTAGCGCCAACGA AGACAAGGCGGCCGACCTTCAGCGCGAAGTCGAGCGCGACGGGGGCC GGTTCAGGTTCGTCGGGCATTTCAGCGAAGCGCCGGGCACGTCGGCGT TCGGGACGGCGGAGCGCCCGGAGTTCGAACGCATCCTGAACGAATGCC GCGCCGGGCGGCTCAACATGATCATTGTCTATGACGTGTCGCGCTTCTC GCGCCTGAAGGTCATGGACGCGATTCCGATTGTCTCGGAATTGCTCGCC CTGGGCGTGACGATTGTTTCCACTCAGGAAGGCGTCTTCCGGCAGGGAA ACGTCATGGACCTGATTCACCTGATTATGCGGCTCGACGCGTCGCACAA AGAATCTTCGCTGAAGTCGGCGAAGATTCTCGACACGAAGAACCTTCAG CGCGAATTGGGCGGGTACGTCGGCGGGAAGGCGCCTTACGGCTTCGAG CTTGTTTCGGAGACGAAGGAGATCACGCGCAACGGCCGAATGGTCAATG TCGTCATCAACAAGCTTGCGCACTCGACCACTCCCCTTACCGGACCCTT CGAGTTCGAGCCCGACGTAATCCGGTGGTGGTGGCGTGAGATCAAGAC GCACAAACACCTTCCCTTCAAGCCGGGCAGTCAAGCCGCCATTCACCCG GGCAGCATCACGGGGCTTTGTAAGCGCATGGACGCTGACGCCGTGCCG ACCCGGGGCGAGACGATTGGGAAGAAGACCGCTTCAAGCGCCTGGGAC CCGGCAACCGTTATGCGAATCCTTCGGGACCCGCGTATTGCGGGCTTCG CCGCTGAGGTGATCTACAAGAAGAAGCCGGACGGCACGCCGACCACGA AGATTGAGGGTTACCGCATTCAGCGCGACCCGATCACGCTCCGGCCGG TCGAGCTTGATTGCGGACCGATCATCGAGCCCGCTGAGTGGTATGAGCT TCAGGCGTGGTTGGACGGCAGGGGGCGCGGCAAGGGGCTTTCCCGGG GGCAAGCCATTCTGTCCGCCATGGACAAGCTGTACTGCGAGTGTGGCG CCGTCATGACTTCGAAGCGCGGGGAAGAATCGATCAAGGACTCTTACCG

	CTGCCGTCGCCGGAAGGTGGTCGACCCGTCCGCACCTGGGCAGCACGA AGGCACGTGCAACGTCAGCATGGCGGCACTCGACAAGTTCGTTGCGGA ACGCATCTTCAACAAGATCAGGCACGCCGAAGGCGACGAAGAGACGTTG GCGCTTCTGTGGGAAGCCGCCCGACGCTTCGGCAAGCTCACTGAGGCG CCTGAGAAGAGCGGCGAACGGGCGAACCTTGTTGCGGAGCGCGCCGAC GCCCTGAACGCCCTTGAAGAGCTGTACGAAGACCGCGCGGCAGGCGCG TACGACGGACCCGTTGGCAGGAAGCACTTCCGGAAGCAACAGGCAGCG CTGACGCTCCGGCAGCAAGGGGCGGAAGAGCGGCTTGCCGAACTTGAA GCCGCCGAAGCCCCGAAGCTTCCCCTTGACCAATGGTTCCCCGAAGAC GCCGACGCTGACCCGACCGGCCCTAAGTCGTGGTGGGGGCGCGCGTC AGTAGACGACAAGCGCGTGTTCGTCGGGCTCTTCGTAGACAAGATCGTT GTCACGAAGTCGACTACGGGCAGGGGGCAGGGAACGCCCATCGAGAAG CGCGCTTCGATCACGTGGGCGAAGCCGCCGACCGACGACGACGAAGAC GACGCCCAGGACGGCACGGAAGACGTAGCGGCGTAG
CRISPRi (Promoter-RiboJ-RBS-dCas9-terminator-promoter-sgRNA targeting sequence-sgRNA scaffold-terminator-ori-AprR-pSG5 rep-pSG5 ds-oriT)	TGTTCACATTCGAACCGTCTCTGCTTTGACATCGTGTGGCGCTTGGGTGT AAAGTCGTGGCCATTAAACAAAATTATTTGTAGAGGCTGTTTCGTCCTCA CGGACTCATCAGACCGGAAAGCACATCCGGTGACAGCTAACTACGAAGG GGAGTCAGTATGGACAAGAAGTACAGCATCGGCCTGGCCATCGGCACC AACAGCGTGGGCTGGGCGGTCATCACCGACGAGTACAAGGTCCCCTCC AAGAAGTTCAAGGTCCTGGGCAACACCGACCGGCACTCGATCAAGAAGA ACCTGATCGGCGCCCTGCTCTTCGACAGCGGCGAAACCGCCGAGGCGA CCCGCCTGAAGCGGACCGCCCGTCGCCGCTACACCCGGCGCAAGAACC GCATCTGCTACCTGCAGGAGATCTTCTCCAACGAGATGGCCAAGGTCGA CGACTCGTTCTTCCACCGGCTCGAGGAGAGCTTCCTGGTGGAGGAGGA CAAGAAGCACGAGCGCCACCCGATCTTCGGCAACATCGTCGACGAGGT GGCCTACCACGAGAAGTACCCCACCATCTACCACCTCCGCAAGAAGCTG GTCGACTCGACCGACAAGGCGGACCTGCGGCTCATCTACCTGGCCCTC GCGCACATGATCAAGTTCCGCGGCCACTTCCTCATCGAGGGCGACCTGA ACCCGGACAACTCCGACGTCGACAAGCTCTTCATCCAGCTGGTGCAGAC CTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTCGA CGCCAAGGCGATCCTCTCCGCGCGCCTGAGCAAGTCCCGGCGCCTGGA GAACCTCATCGCCCAGCTGCCGGGCGAGAAGAAGAACGGCCTCTTCGG CAACCTGATCGCGCTGTCGCTCGGCCTGACCCCCAACTTCAAGAGCAAC TTCGACCTGGCCGAGGACGCGAAGCTCCAGCTGTCCAAGGACACCTAC GACGACGACCTGGACAACCTGCTCGCCCAGATCGGCGACCAGTACGCG GACCTCTTCCTGGCCGCGAAGAACCTCTCGGACGCCATCCTGCTCAGCG ACATCCTGCGGGTCAACACCGAGATCACCAAGGCCCCGCTGTCGGCGA GCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCTGCTCAA GGCCCTCGTGCGCCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCTTC GACCAGTCCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCGTCG CAGGAGGAGTTCTACAAGTTCATCAAGCCGATCCTGGAGAAGATGGACG GCACCGAGGAGCTGCTCGTCAAGCTGAACCGCGAGGACCTGCTCCGCA AGCAGCGGACCTTCGACAACGGCTCCATCCCGCACCAGATCCACCTGG GCGAGCTCCACGCCATCCTCCGGCGCCAGGAGGACTTCTACCCCTTCCT GAAGGACAACCGCGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCC GTACTACGTCGGCCCCCTGGCCCGCGGCAACTCCCGGTTCGCGTGGAT GACCCGGAAGTCGGAGGAAACCATCACCCCGTGGAACTTCGAGGAGGT CGTGGACAAGGGCGCCTCCGCGCAGTCGTTCATCGAGCGCATGACCAA CTTCGACAAGAACCTCCCGAACGAGAAGGTCCTGCCCAAGCACAGCCTG CTCTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTCAAGTACG TGACCGAGGGCATGCGGAAGCCGGCCTTCCTGTCCGGCGAGCAGAAGA

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TTGGGGCGGAGTCTCACCAAGGACTACGTCTGCTGGCGATTTCCGTTA
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GGGGTCATTATAGCGATTTTTTCGGTATATCCATCCTTTTTCGCACGATA
TACAGGATTTTGCCAAAGGGTTCGTGTAGACTTTCCTTGGTGTATCCAA
CGGCGTCAGCCGGGCAGGATAGGTGAAGTAGGCCCACCCGCGAGCGG
GTGTTCCTTCTTCACTGTCCCTTATTCGCACCTGGCGGTGCTCAACGGG
AATCCTGCTCTGCGAGGCTGGCCGGCTACCGCCGGCGTAACAGATGAG
GGCAAGCGGATGGCTGATGAAACCAAGCCAACCAGGAAGGGCAGCCC
ACCTATCAAGGTGTACTGCCTTCCAGACGAACGAAGAGCGATTGAGGA
AAAGGCGGCGGCGGCCGGCATGAGCCTGTCGGCCTACCTGCTGGCCG
TCGGCCAGGGCTACAAAATCACGGGCGTCGTGGACTATGAGCACGTCC
GCGAGCTGGACAGCGTGCAGGACTGGGGGAGTTA

Supplementary table 3. Promoters used in this study. Plasmid sequences can be constructed by replacing the yellow regions in the example plasmids shown above. Promoter sequences were derived from Bai et al.[1], Myronovskyi and Luzhetskyy[2], and Phelan et al[3].

Promoter ID	Sequence (5' to 3')
57	TTGAACGGCTGGAGGGATACACCTGGTCATAGGATACCATC
ermE*p	CTCTAGTATGCATGCGAGTGTCCGTTCGAGTGGCGGCTTGCGCCCGATGCTAG TCGCGGTTGATCGGCGATCGCAGGTGCACGCGGTCGATCTTGACGGCTGGCG AGAGGTGCGGGGAGGATCTGACCGACGCGGTCCACACGTGGCACCGCGATGC TGTTGTGGGCACAATCGTGCCGGTTGGTAGGATCCACAT
gapdh(EL)	GCTGCTCCTTCGGTCGGACGTGCGTCTACGGGCACCTTACCGCAGCCGTCGG CTGTGCGACACGGACGGATCGGGCGAACTGGCCGATGCTGGGAGAAGCGCGC TGCTGTACGGCGCGCACCGGGTGCGGAGCCCCTCGGCGAGCGGTGTGAAACT TCTGTGAATGGCCTGTTCGGTTGCTTTTTTTATACGGCTGCCAGATAAGGCTTG CAGCATCTGGGCGGCTACCGCTATGATCGGGGCGTTCCTGCAATTCTTAGTGC GAGTATCTGAAAGGGGATACGC
KasO*p	TGTTCACATTCGAACGGTCTCTGCTTTGACAACATGCTGTGCGGTGTTGTAAAG TCGTGGCC
rpsl(XC)	GCCCTGCAGGCGGAAGTCAGGTAGACACGACTTCCGCTAGTCCTTGCAAGGTC TGCTGACGTGAGGCGGGGCGGTCGTTTTTGACCGCCCTGCCTTCGTCATGTAG GCTCGCTCGCTGTGCCTGGCGTGTCATCAGACGCCCAGGTCCCGGTGCCGTG AGGCCCGGGCCATCGAGCCGGTGGTACGTGGCTGCGGTCCCCTTGTGAGGGC TGCGCGCCGTGTGCTGTCCGGCGCGCACAGCCTTGAATCCACCCGCGGGGGC CGGCCGGTCTCCGTGAGCTCGAGTAGACGACGGAGACGTA
SP1	TGTTCACATTCGAACCGTCTCTGCTTTGACATGGAGAGAAGTTTTGTAAAGTCG TGGCCA
SP10	TGTTCACATTCGAACCGTCTCTGCTTTGACATGTTCTTACGGTCACATGTAAAGT CGTGGCCA
SP20	TGTTCACATTCGAACCGTCTCTGCTTTGACACATGACGCTCACCCGTTGTAAAG TCGTGGCCA
SP30	TGTTCACATTCGAACCGTCTCTGCTTTGACATCGTGTGGCGCTTGGGTGTAAAG TCGTGGCCA
SP43	TGTTCACATTCGAACCGTCTCTGCTTTGACACGGACAAGCGCTATGGTGTAAAG TCGTGGCCA

Supplementary table 4. sgRNA sequences used in this study. Plasmid sequences can be constructed by replacing the dark yellow regions in the example plasmids shown above.

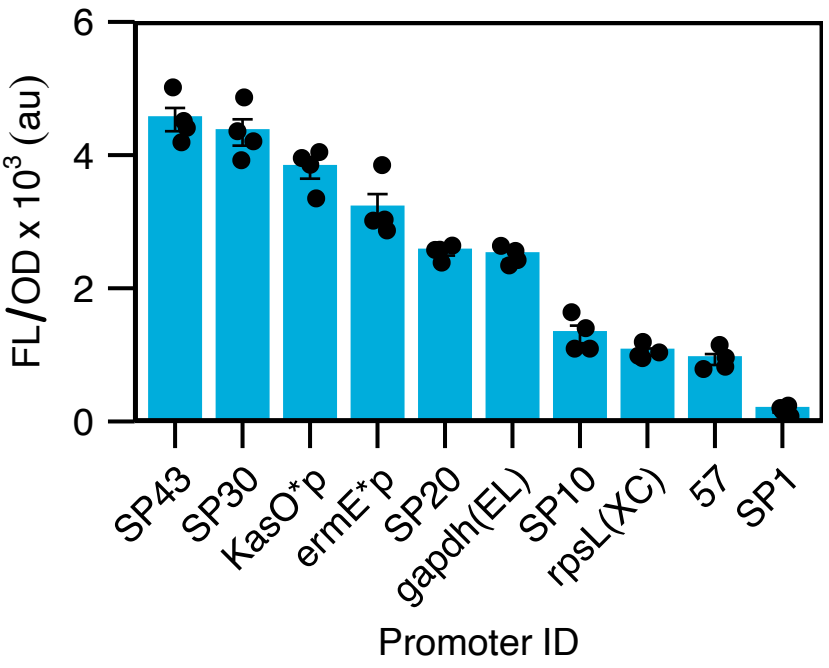
Sequence (5' to 3')	PAM location	Strand	Target	Type	Tool	Plasmid
CATGTTG TCCTCCT CGCCCT	11	NT	mCherry	On-target	CRISPRi	pJEC711, pJEC712, pJEC713, pJEC714
TCTGGGT GCCCTCG TACGGC	123	NT	mCherry	On-target	CRISPRi	pJEC727
GTCGGCC GGGTGCT TGACGT	230	NT	mCherry	On-target	CRISPRi	pJEC728

TCTTGACTTCGGCGTCGTAG	531	NT	mCherry	On-target	CRISPRi	pJEC729
CGTGTAGTCCTCGTTGTGCG	623	NT	mCherry	On-target	CRISPRi	pJEC730
CAAGGGCGAGGAGGACAACA	29	T	mCherry	On-target	CRISPRi	pJEC731
GGGGAGGGCCGGCCGTACGA	132	T	mCherry	On-target	CRISPRi	pJEC732
GAAGGCCTACGTCAAGCACC	242	T	mCherry	On-target	CRISPRi	pJEC733
CGAAGTCAAGACGACGTACA	560	T	mCherry	On-target	CRISPRi	pJEC734
CAACGAGGACTACACGATCG	647	T	mCherry	On-target	CRISPRi	pJEC735
GGCTCAGGTGAAGAGCGGGG	NA	NT	Non-coding genomic sequence	Off-target	CRISPRi, CRISPRa	pJEC723, pJEC737, pJEC740, pJEC743
GCGTGCGTCGCACCTCCGTG	NA	NT	Non-coding genomic sequence	Off-target	CRISPRi	pJEC724
TGCACTGCTGTAAGGACGAT	NA	NA	No match	Off-target	CRISPRi	pJEC725
CCGTGTGGCCCGCTCTTGTT	44	NT	jadR2	On-target	CRISPRi	pJEC749
AGGTATCCTGCGGTGTCCTG	-82	T	mCherry	On-target	CRISPRa	pJEC739, pJEC742, pJEC745
AGGACGCCTTTGGTAACCGC	-83	NT	mCherry	On-target	CRISPRa	pJEC738, pJEC741, pJEC744
ATGTGAACAAAGGACGCCTT	-73	NT	mCherry	On-target	CRISPRa	pJEC747
TGGTAACCGCAGGACACCGC	-93	NT	mCherry	On-target	CRISPRa	pJEC748
CGTCAGAATTCACAAGCCCG	-73	NT	jadJ	On-target	CRISPRa	pJEC750

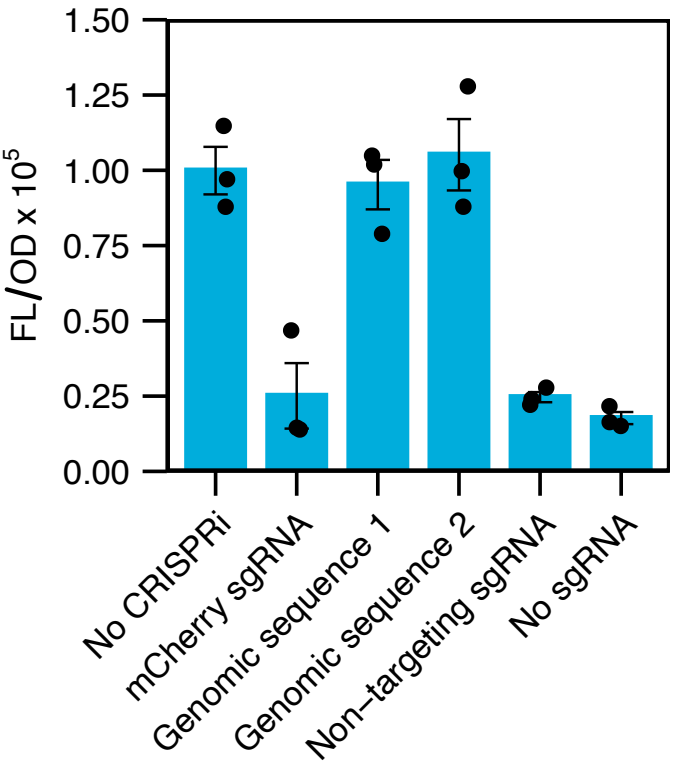
Supplementary table 5. Activator domains (ADs) used in this study. Plasmid sequences can be constructed by replacing the dark green regions in the example plasmids shown above.

AD	Nucleotide sequence	Protein sequence	Plasmids
αNTD	ATGCTTATCGCTCAGCGTCCTTCGCTGA CCGAAGAGGTCGTCGACGAGTTCCGCT CCCGGTTCGTGATCGAGCCGCTGGAGC CGGGCTTCGGCTACACCCTCGGCAACTC CCTCCGCCGTACCCTCCTCTCCTCGATC CCGGGTGCCGCTGTCACCAGCATCCGC ATCGACGGTGTCCTGCACGAGTTCACCA CCGTGCCGGGCGTCAAGGAGGACGTCA CCGACCTCATCCTCAACATCAAGCAGCT GGTCGTCTCCTCGGAGCACGACGAGCC GGTCGTGATGTACCTGCGCAAGCAGGG CCCGGGTCTGGTCACCGCCGCCGACAT CGCGCCCCCGGCCGGTGTCGAGGTGCA CAACCCCGACCTCGTCCTCGCCACGCTC AACGGCAAGGGCAAGCTGGAGATGGAG CTGACCGTCGAGCGCGGTCGCGGCTAC GTCTCCGCCGTGCAGAACAAGCAGGTC GGTCAGGAGATCGGGCGCATCCCGGTC GACTCGATCTACTCGCCGGTTCTCAAGG TCACCTACAAGGTCGAGGCGACCCGAGT CGAGCAGCGCACCGACTTCGACAAGCT GATCGTCGACGTCGAGACCAAGCAGGC CATGCGCCCGCGTGACGCCATGGCGTC CGCCGGCAAGACCCTGGTCGAGCTGTT CGGTCTGGCGCGCGAGCTCAACATCGA CGCC	MLIAQRPSLTEEVVDE FRSRFVIEPLEPGFGY TLGNSLRRTLLSSIPGA AVTSIRIDGVLHEFTTV PGVKEDVTDLILNIKQL VVSSEHDEPVVMYLR KQGPGLVTAADIAPPA GVEVHNPDLVLATLNG KGKLEMELTVERGRG YVSAVQNKQVGQEIG RIPVDSIYSPVLKVTYK VEATRVEQRTDFDKLI VDVETKQAMRPRDAM ASAGKTLVELFGLARE LNIDA	pJEC737, pJEC738, pJEC739, pJEC747, pJEC748, pJEC750
ω	GTGTCCTCTTCCATCACCGCGCCCGAGG GCATCATCAACCCGCCGATCGACGAGCT GCTCGAGGCCACGGACTCGAAGTACAG CCTCGTGATCTACGCGGCCAAGCGCGC GCGTCAGATCAACGCGTACTACTCCCAG CTCGGCGAGGGCCTGCTGGAGTACGTG GGTCCGCTGGTCGACACCCACGTCCAC GAGAAGCCGCTCTCGATCGCCCTGCGC GAGATCAACGCGGGCCTGCTGACGTCC GAGGCCATCGAGGGCCCGGCGCAG	VSSSITAPEGIINPPIDE LLEATDSKYSLVIYAAK RARQINAYYSQLGEGL LEYVGPLVDTHVHEKP LSIALREINAGLLTSEAI EGPAQ	pJEC740, pJEC741, pJEC742
RbpA	ATGAGTGAGCGAGCTCTTCGCGGCACG CGCCTCGTGGTGACGAGCTACGAGACC GACCGCGGCATCGATCTGGCCCCGCGC CAGGCCGTGGAGTACGCATGCGAGAAG GGCCATCGTTTTGAGATGCCCTTCTCGG TGGAAGCGGAAATTCCGCCGGAGTGGG AGTGCAAGGTCTGCGGAATCCAGGCACT CCTGGTGGACGGGGACGGACCTGAGGA	MSERALRGTRLVVTSY ETDRGIDLAPRQAVEY ACEKGHRFEMPFSVE AEIPPEWECKVCGIQA LLVDGDGPEEKKGKP ARTHWDMLMERRTRE ELEEVLAERLAVLRSG AMNIAVHPRDSRKSA	pJEC743, pJEC744, pJEC745

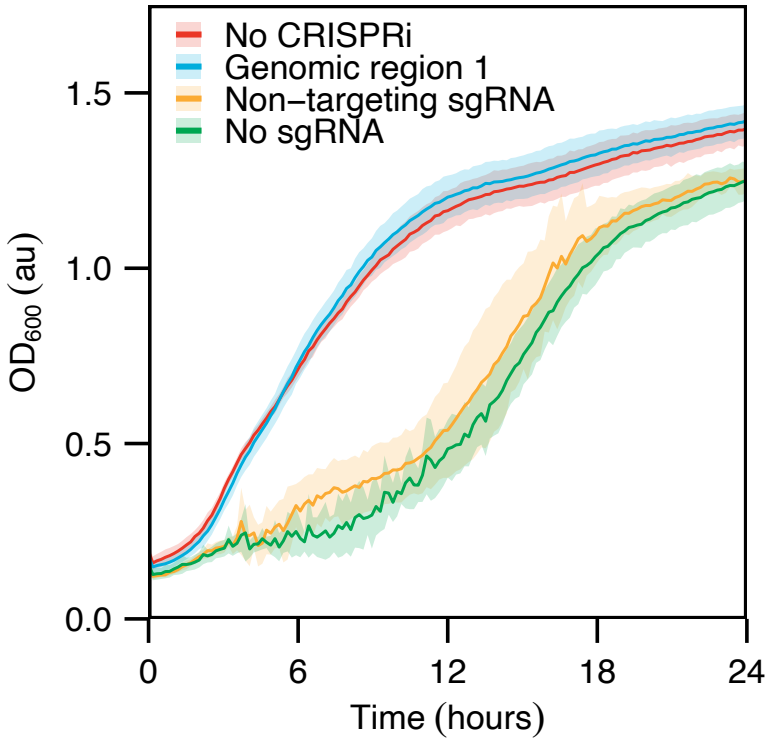
	GAAGAAGGGCAAGCCTGCGCGTACGCA CTGGGACATGCTCATGGAGCGACGCAC CCGCGAGGAGCTGGAGGAGGTCCTCGC CGAAAGGCTGGCCGTCCTGCGTTCCGG CGCCATGAACATCGCCGTGCATCCGCG CGACAGCCGCAAGTCCGCC		



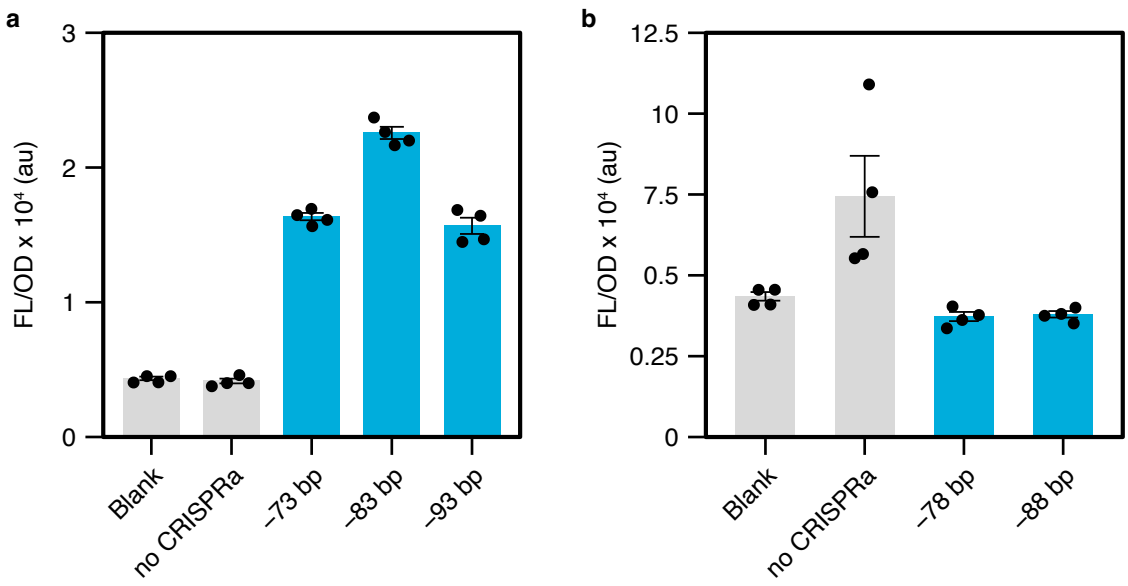
Supplementary figure 1. Evaluating the strength of a library of *Streptomyces* promoters. Fluorescence characterization of constitutive promoters. The expression strength of a constitutive promoter library was evaluated by cloning each promoter upstream of an mCherry reporter and integrating the resulting reporter constructs into the genome of *S. venezuelae* cells at the ΦC31 attB site. Fluorescence characterization was performed by bulk fluorescence measurements (measured in units of fluorescence [FL]/optical density [OD] at 600 nm). Data represent mean values and errors bars represent standard deviation of 4 biological replicates.



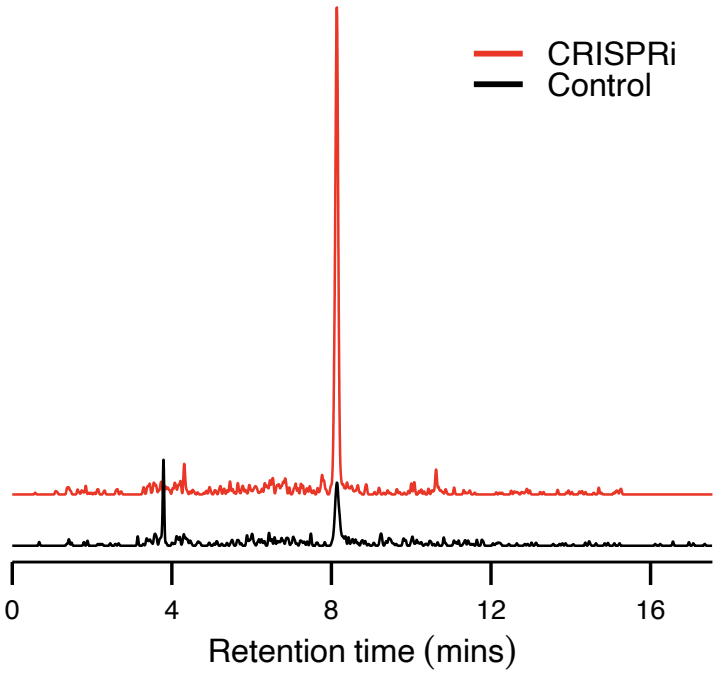
Supplementary figure 2. CRISPRi results in decreased fluorescence in the absence of a sgRNA or in the presence of non-targeting sgRNA. Fluorescence characterization of *S. venezuelae* cells containing a genomically-integrated mCherry gene conjugated with CRISPRi plasmids containing different sgRNAs or a no CRISPRi control plasmid. mCherry sgRNA binds to the mCherry gene and represses expression. Genomic sequence 1 and 2 are sgRNAs designed to non-coding sequences present in the *S. venezuelae* genome. Non-targeting sgRNA is designed to target a sequence absent in the *S. venezuelae* genome. No sgRNA is a CRISPRi plasmid without an sgRNA. Fluorescence characterization was performed by bulk fluorescence measurements (measured in units of fluorescence [FL]/optical density [OD] at 600 nm). Data represent mean values and errors bars represent standard deviation of at least 3 biological replicates.



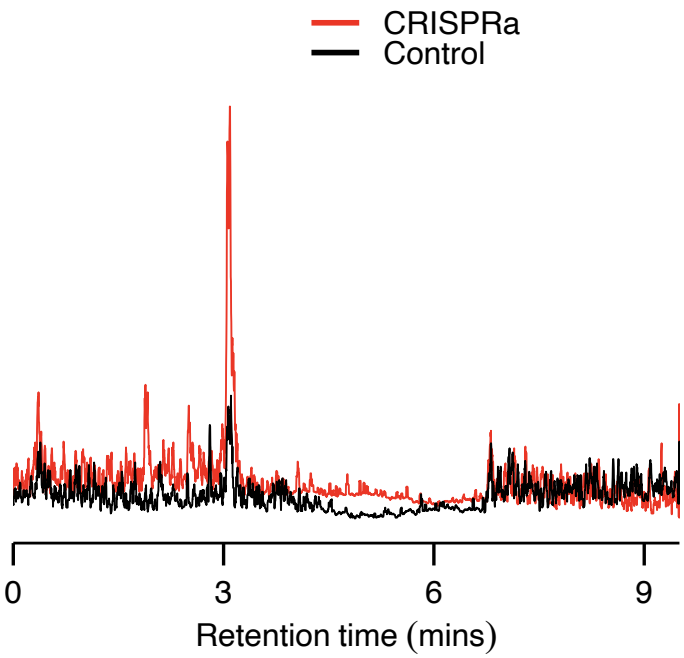
Supplementary figure 3. CRISPRi results in inhibition of growth in the absence of a sgRNA or in the presence of non-targeting sgRNA. Growth measurement of *S. venezuelae* conjugated with CRISPRi plasmids containing different sgRNAs or a no CRISPRi control plasmid. mCherry sgRNA binds to the mCherry gene and represses expression. Genomic region is a sgRNA designed to a non-coding sequence present in the *S. venezuelae* genome. Non-targeting sgRNA is designed to target a sequence absent in the *S. venezuelae* genome. No sgRNA is a CRISPRi plasmid without an sgRNA. Growth was evaluated by measuring optical density at 600 nm (OD_{600}) every 10 minutes at 30 °C with 90 rpm shaking. Lines represent mean values over time and shaded area depicts standard deviation of 4 biological replicates.



Supplementary figure 4. Evaluating distance-dependent activation patterns of CRISPRa. Fluorescence characterization of *S. venezuelae* cells containing a genomically-integrated mCherry gene conjugated with CRISPRa plasmids containing different sgRNAs or a no CRISPRa control plasmid. Blank cells are *S. venezuelae* cells lacking mCherry and transformed with the no CRISPRa plasmid that are used to determine autofluorescence. In (a) a reporter containing PAMs on the template strand at 73, 83, and 93 bp upstream of the reporter promoter's TSS is used. In (b) an additional 5 bp are added before the reporter promoter's TSS to create PAMs at 78 and 88 bp upstream. Fluorescence characterization was performed by bulk fluorescence measurements (measured in units of fluorescence [FL]/optical density [OD] at 600 nm). Data represent mean values and errors bars represent standard deviation of at least 4 biological replicates



Supplementary figure 5. Activating production of jadomycin B using CRISPRi. LC-MS analysis of crude extracts of *S. venezuelae* cells conjugated with CRISPRi plasmids designed to repress the expression of jadR2. Cells were cultured, fermented, and extracted as described in methods. The crude extracts were then analyzed via liquid chromatography coupled to mass spectrometry (LC-MS). The reported data are extracted ion chromatogram at the corresponding *m/z* value of jdB (m/z = 550.2059, $[M+H]^+$). A jdB standard was run in parallel, and showed the same elution time as the CRISPRi sample. We note that the observed elution time of these replicates differs from the data shown in Figure 3c due to different instrumentation. For these samples, LC-MS analysis was carried out using a Shimadzu IT-ToF MS system interfaced to a Shimadzu LC-20 ADXR LC system through an Electro Sprat Ionization (ESI) source. Separations were carried out using an Ascentis Express C18 column (1.0 mm ID x 150 mm with 90A, 2.7u particles). The LC was operated at a flow rate of 0.175 mL/min with mobile phase A (0.1% Formic Acid in Water) and B (0.1% Formic Acid in Acetonitrile). The Analytical separation was carried out over 10 min; going from 10%B (t= 0 min) to 95% B(t= 10 min).



Supplementary figure 6. Activating production of jadomycin B using CRISPRa. LC-MS analysis of crude extracts of strains harboring jadJ-V-targeting CRISPRa plasmids. To induce the production of jdB, a sgRNA was designed to target a sequence downstream of a PAM site at 73 bp from the TSS of the jadJ-V operon within the jdB BGC. The sgRNA was cloned into a plasmid harboring the αNTD-based CRISPRa system. The plasmid was conjugated into wild-type *S. venezuelae*, and the resulting strains were cultured, fermented, and extracted. The crude extracts were then analyzed via liquid chromatography coupled to mass spectrometry (LC-MS), as described in the methods. The reported data are extracted ion chromatogram at the corresponding m/z value of jdB (m/z = 550.2059, $[M+H]^+$) for a second set of representative biological replicates (other than the ones shown in Figure 3d). A jdB standard was run in parallel, and showed the same elution time as the CRISPRa sample.

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