SUPPLEMENTARY FIGURES AND TABLE

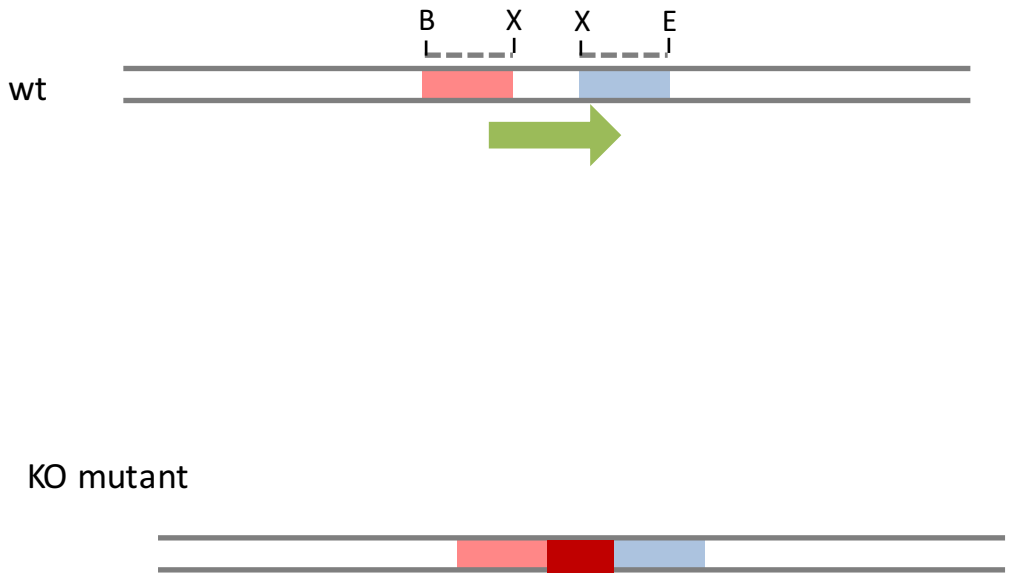

Figure S1. Scheme of gene knockouts, Related to STAR Methods. The target *pum* gene is indicated by a green arrow: two fragments of ~1.5-kbp each were amplified from genomic DNA (wt) using primers containing *BamHI* (B) and *XbaI* (X) and *XbaI* and *EcoRI* (E) tails (Table S1). In the knockout (KO) mutant the target *pum* gene is replaced by the apramycin resistance gene (in red).

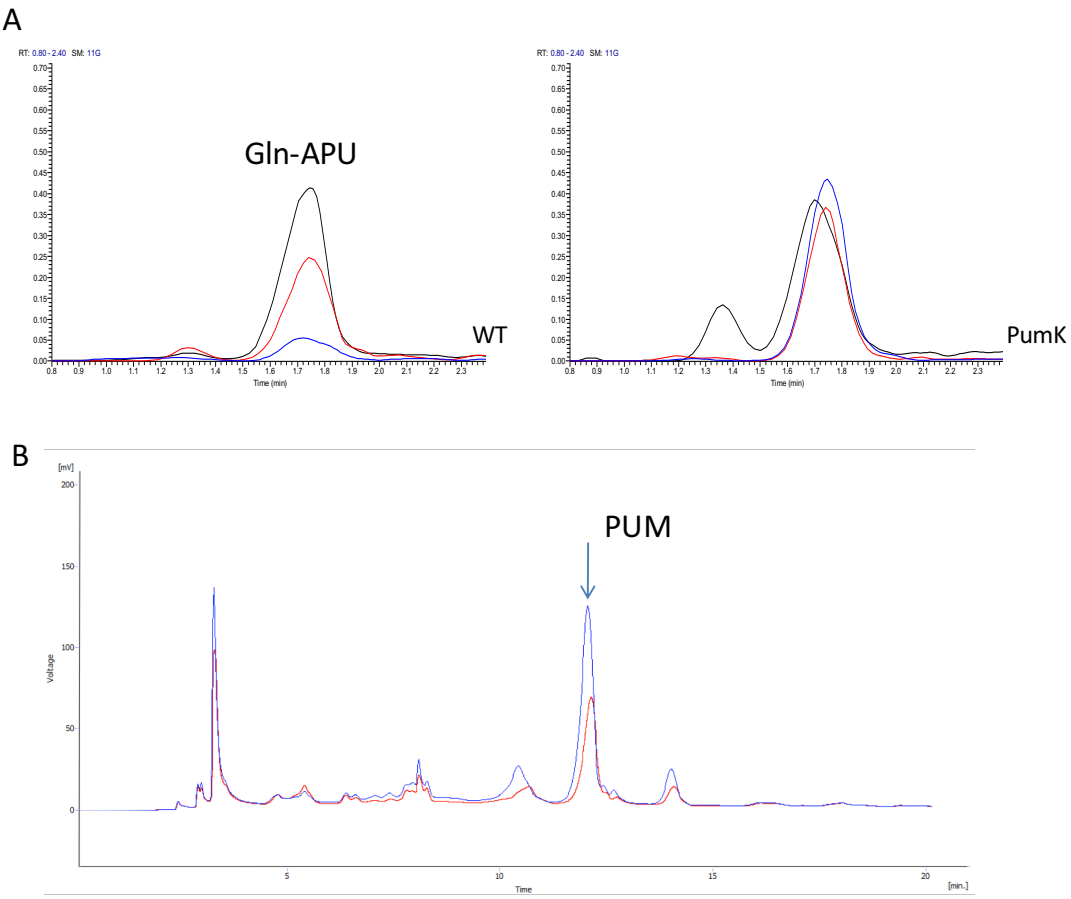

Figure S2. Addition of Gln-APU (Panel A) Related to Figure 3, and of guanidinoacetic acid (Panel B) Related to Figure 4. (A) Extracted ion chromatograms of Gln-APU (*m/z* 372 $[M+H]^+$) at 24h (black line), 48h (red line) and 72h (blue line) from duplicate cultures of the parental strain (WT) and of the *pumK* mutant (PumK) grown in medium supplemented with Gln-APU. (B) HPLC profile of *Streptomyces* sp. ID38673 culture extract without (red line) and with (blue line) 0.5 mg/mL guanidinoacetic acid. The PUM peak is indicated. Samples were analyzed as described in STAR Method with detector set at 262nm.

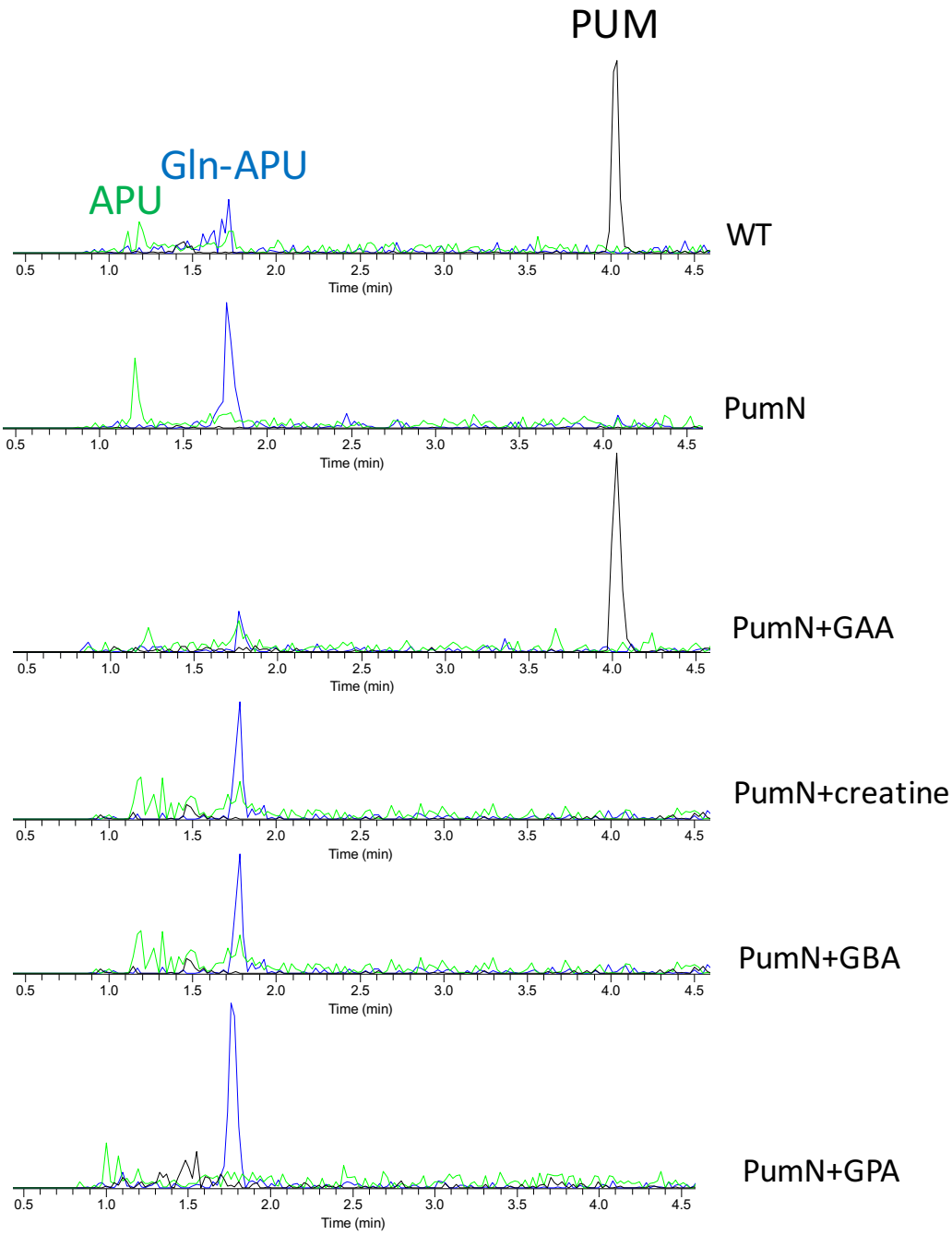

Figure S3. Supplementation of the *pumN* mutant, Related to Figure 4. Analyses were performed on cultures of the parental strain (WT), and of the pumN mutant without (PumN), and with (PumN + GAA, PumN+ creatine, PumN + GBA and PumN+ GPA) supplementation with 0.5 mg/mL guanidinoacetic acid (GAA), creatine, guanidinobutyric acid (GBA) and guanidinopropionic acid (GPA), respectively. The analyses show extracted ion chromatograms of pseudouridimycin (PUM, *m/z* 487 $[M+H]^+$, black line), aminopseudouridine (APU, *m/z* 244 $[M+H]^+$, green line) and Gln-APU (*m/z* 372$[M+H]^+$, blue line).

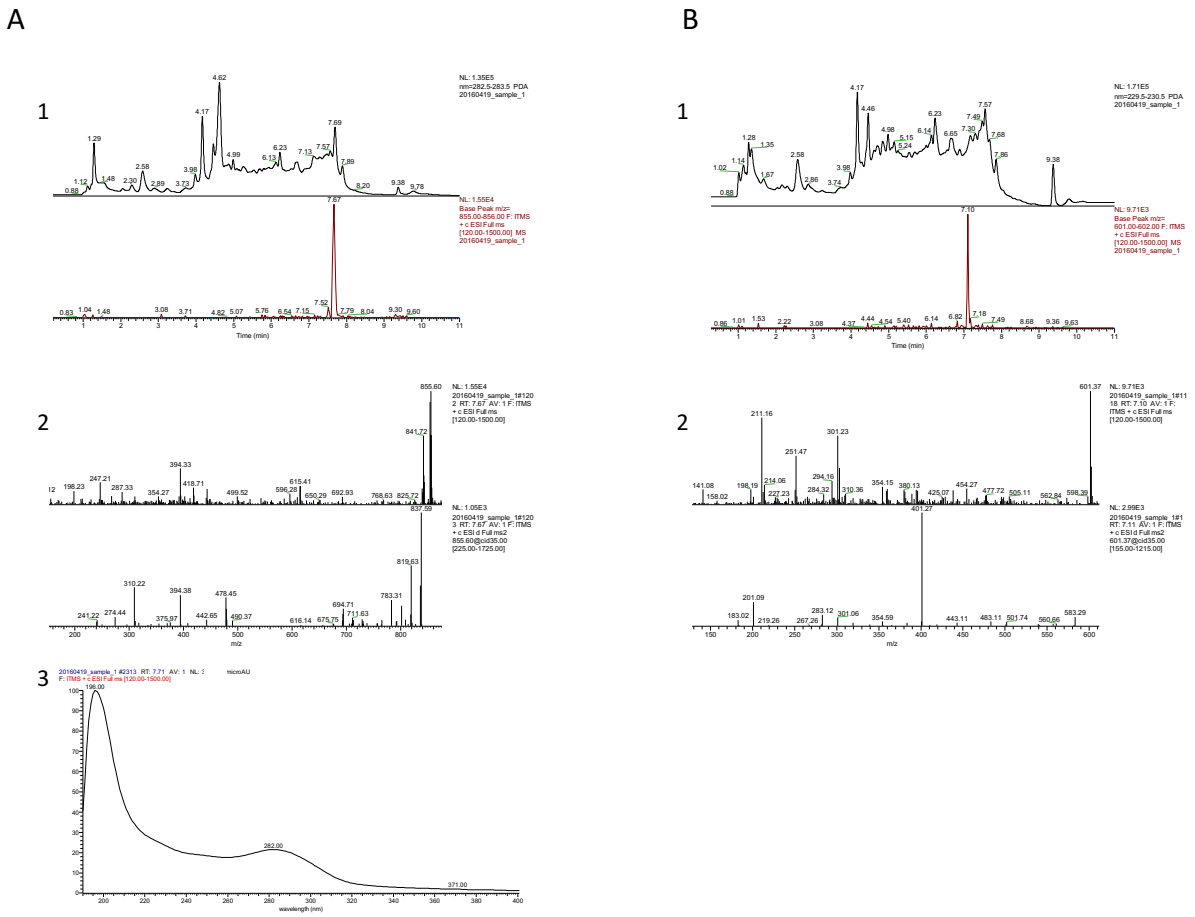

Figure S4. Identification of lydicamycin and desferrioxamine, Related to STAR Methods.

(A) Lydicamycin. 1. HPLC trace at 283 nm of a cell-free broth of the WT strain (top) and extracted ion chromatogram of *m/z* 855 $[M+H]^+$ (bottom); 2. MS at 7.67 min and MS/MS of the ion at *m/z* 855 $[M+H]^+$; 3. total UV-vis spectrum of the 7.67-min peak. (B) Desferrioxamine. 1. HPLC trace at 230 nm of a cell-free broth of the WT strain and extracted ion chromatogram of *m/z* 601 $[M+H]^+$; 2. MS at 7.10 min and MS/MS spectrum of the ion at *m/z* 601 $[M+H]^+$.

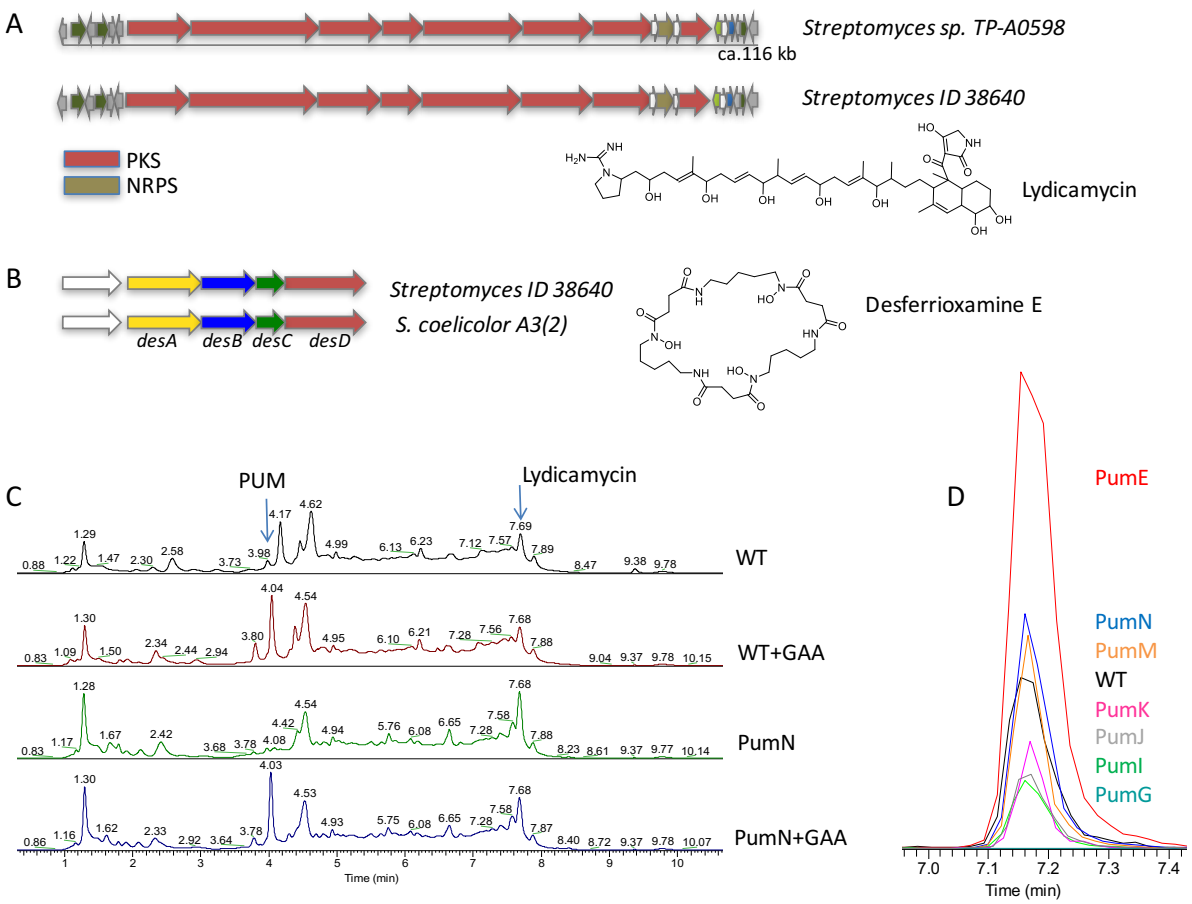

Figure S5. The lydicamycin and desferrioxamine B biosynthetic gene clusters (Panel A and B), Related to STAR Methods. Effect of pum mutations on lydicamycin and desferroxiamine (Panel C and D), Related to Figure 1-4.

(A) Organization of the lydicamycin biosynthetic cluster of *Streptomyces* sp. TP-A0598 (Komaki et al., 2015) and of *Streptomyces* sp. ID38640. The structure of lydicamycin is shown below. (B) Organization of the desferrioxamine B biosynthetic cluster of *S. coelicolor* A3(2) (Barona-Gómez et al., 2004) and of *Streptomyces* sp. ID38640. The structure of desferrioxamine B is shown below. (C) LC analysis of the parental strain (WT), and of the pumN mutant without (PumN), and with (PumN + GAA) supplementation with 0.5 mg/mL guanidinoacetic acid. The figure shows the extracted ion chromatograms of pseudouridimycin (PUM, m/z 487 [M+H]+, black line). (D) Extracted ion chromatograms at 7.1 min (m/z 601 [M+H]+) showing desferrioxiamine levels in the parental strain (WT) and in the pum mutants.

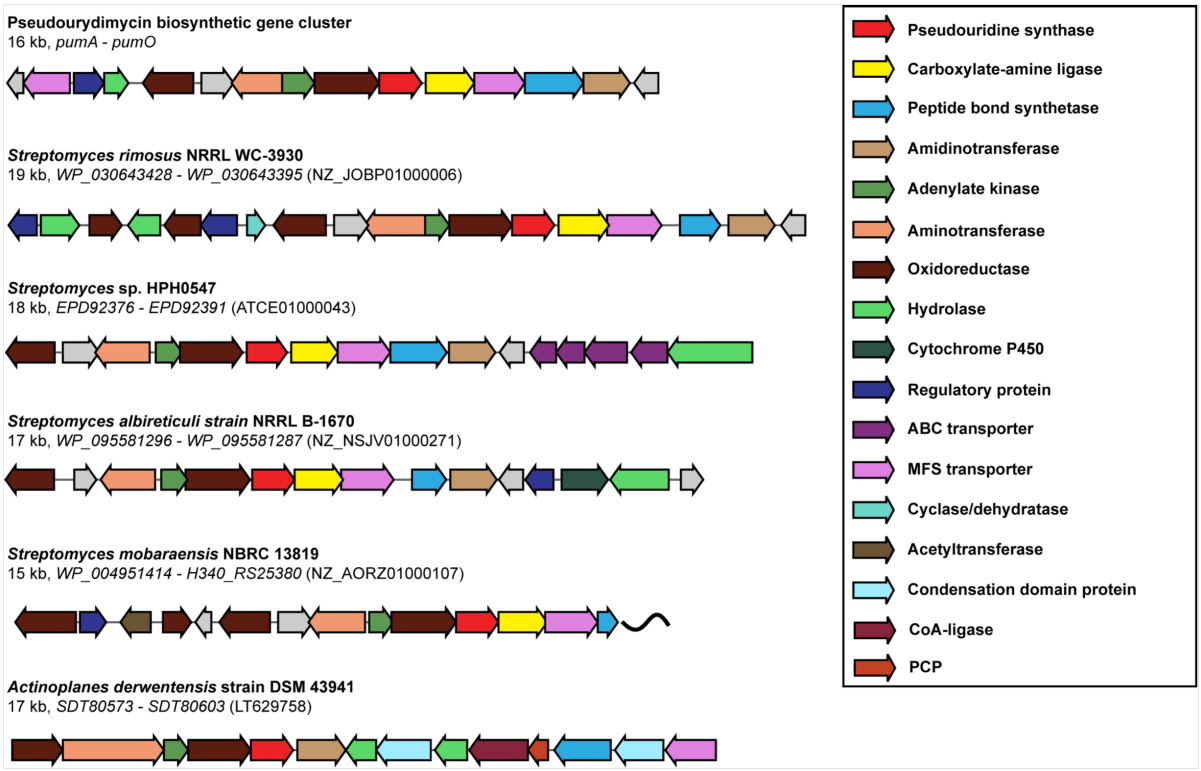

Figure S6. Organization of family 1 BGCs, Related to Figure 5. Selected BGCs from Figure 5 are reported, i.e. one subspecies for *S. rimosus* and *S. albireticuli*. The predicted functions of the CDSs are color-coded as in the side panel.

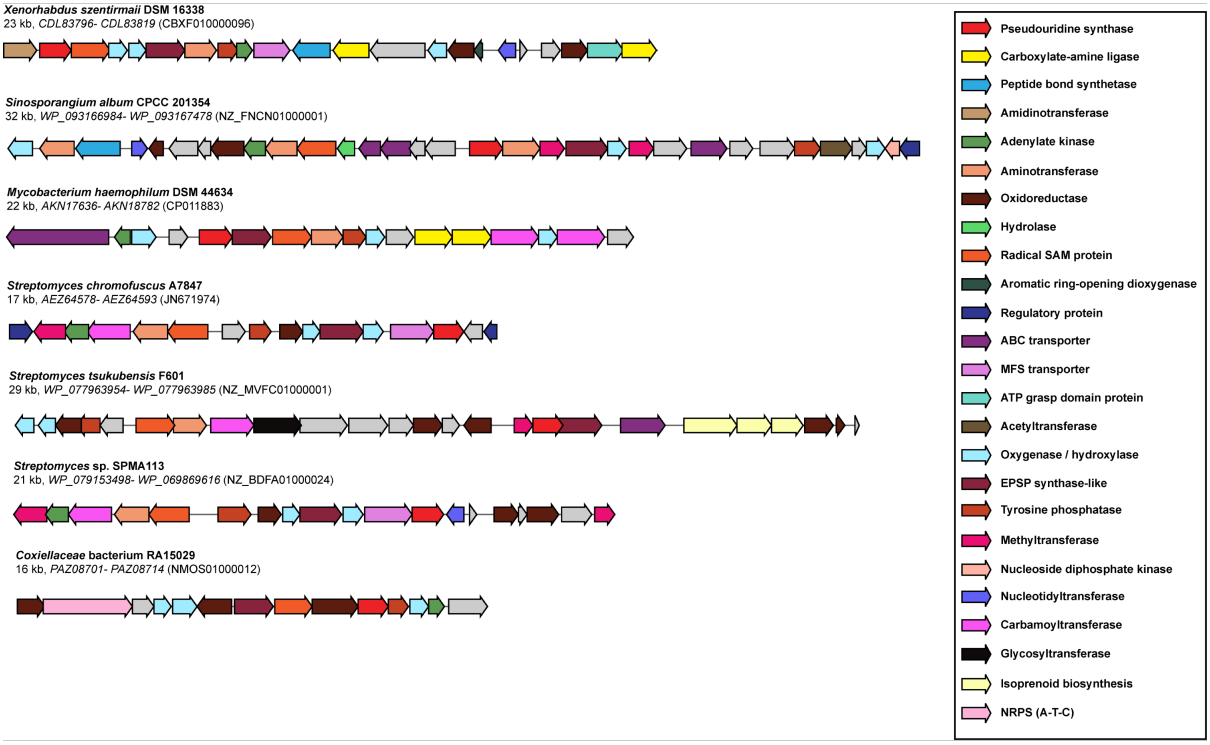

Figure S7. Organization of family 2 BGCs, Related to Figure 5. Selected BGCs from Figure 5 are reported. The predicted functions of the CDSs are color-coded as in the side panel.

Supplementary Table S1. List of primers used for knock out experiments, Related to STAR Methods

gene	fragment[a]	Sequence (5'-3')
pumE	A	TTTGAATTCGACCTCTTCGGCGTCATC
		TTTTCTAGAGACACCACGCTGGGCCTG
	B	TTTTCTAGAAATCGACATGATGCCTCCACGTT
		TTTAGATCTTGCGGACCTGTGACCTG
pumI	A	TTTGAATTCATCGCTGACGCCATCGC
		TTTTCTAGAGAAGCCCTGACGCATCGGTA
	B	TTTTCTAGAGGCAAGTGACCGGCACCCT
		TTTGGATCCGAGGGCAACAGGAAGGAGAC
pumJ	A	TTTGAATTCCGTGGCCGACCACAACG
		TTTTCTAGACAGGGTGCCGGTCACTTGCCTC
	B	TTTTCTAGACGGCAGTTGGTCCTCCGCC
		TTTGGATCCGCCGCTGCGAGCAT
pumK	A	TTTGAATTCAAGCACCGCCAGGAGGAC
		TTTTCTAGAGCCGGGAGCAGGGGAAG
	B	TTTTCTAGACGATGGGCGGCACCGTGA
		TTTGGATCCGAGGGAGACCGCGACCA
pumM	A	TTTGAATTCCTGGGCAGCGGCGTCTGG
		TTTTCTAGACCGCCTGGAGGTGCTGGTCAT

	B	TTTTCTAGACGCACGGAAACAGCGCAC
		TTTAGATCTTCTCCTGGGCCTCGCAGAC
pumN	A	TTTGAATTCTCGAGGCGTGGCTG
		TTTTCTAGAATTGACCAGGCTCACC
	B	TTTTCTAGATTTGCCGCTGGTTACCGG
		TTTGGATCCTCCTCGCGGTGTGCGGC
aac(3)IV [b]	A	TTTTCTAGAGTTCATGTGCAGCTCCATCAG
	B	TTTTCTAGACAGCAATCAGCGCGACCTTG
tsr [b]	A	CGGGGATCGACCGCGCGGGT
	B	AGTGCCCGCCCGGACCACGA

[a] see STAR methods for details and Figure S1; [b] primers used for PCR analysis of the ex-conjugants. *aac(3)IV,* apramycin resistance gene, *tsr,* thiostrepton resistance gene