Cell Reports, Volume *42*

Supplemental information

Pyochelin biotransformation by *Staphylococcus aureus* shapes bacterial competition with *Pseudomonas aeruginosa* in polymicrobial infections

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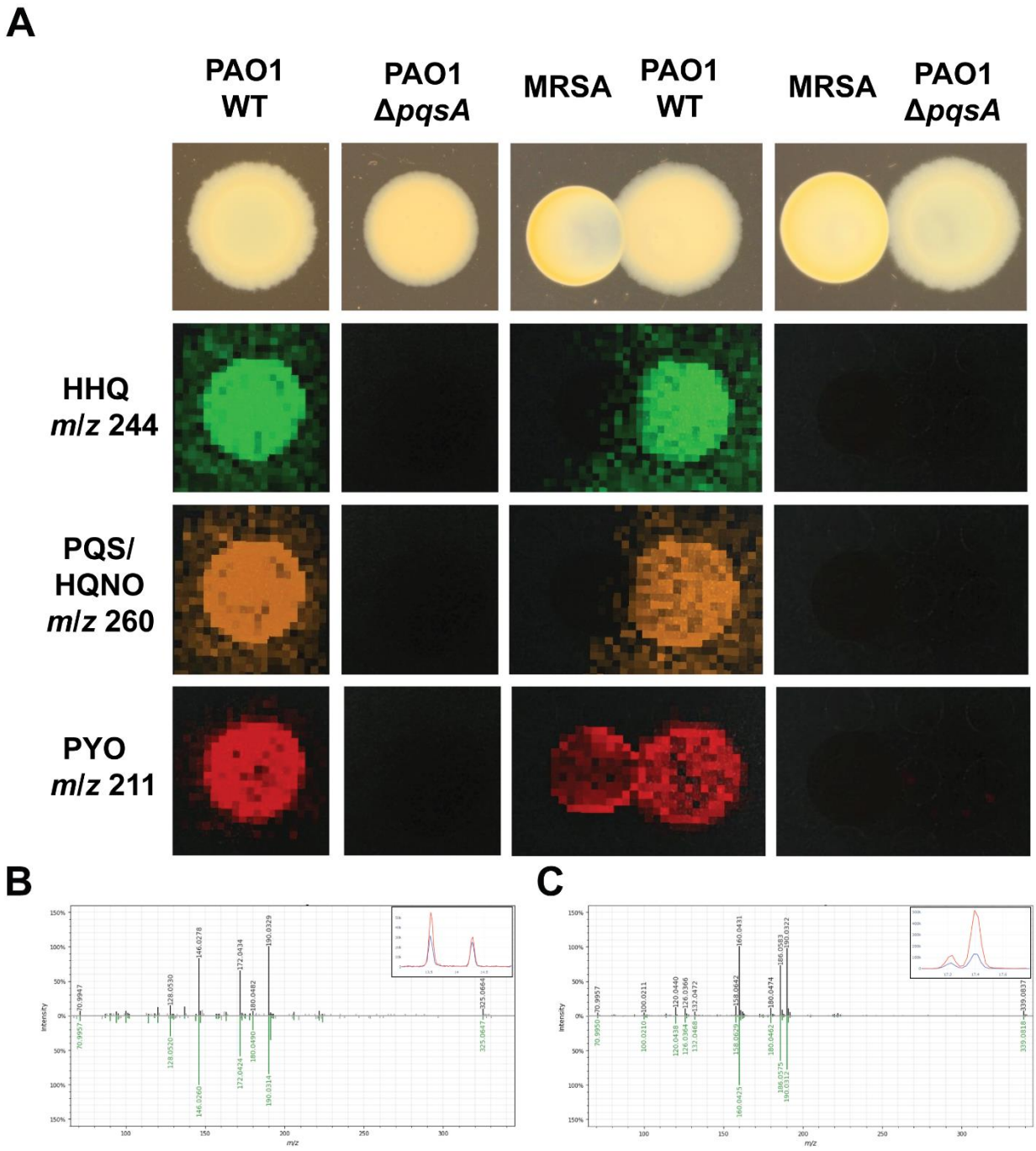

Figure S1: *P. aeruginosa* derived quinolones and pyocyanin drive killing of *S. aureus*. Related to Figure 1. **A**) MALDI-MSI of *S. aureus* and *P. aeruginosa* grown as monocultures or interactions. Photographs of the cultures are shown on top. The second to fourth rows show the false colored *m/z* distribution for HHQ (*m/z* 244), PQS/HQNO

(*m/z* 260) and pyocyanin (PYO; *m/z* 211). *P. aeruginosa* wildtype (PAO1 WT) but not a *P. aeruginosa* quinolone mutant (PAO1 Δ*pqsA*) produces the alkyl-quinolones HHQ and PQS/HQNO as well as the quinolone regulated metabolite PYO. **B and C)** Mirror plots comparing the MS^2 spectrum of isolated (top; black trace) and synthetic (bottom; green trace) **B)** pyochelin and **C)** pyochelin methyl ester. Chromatogram traces of isolated (blue) and synthetic (red) metabolites are shown as inlays.

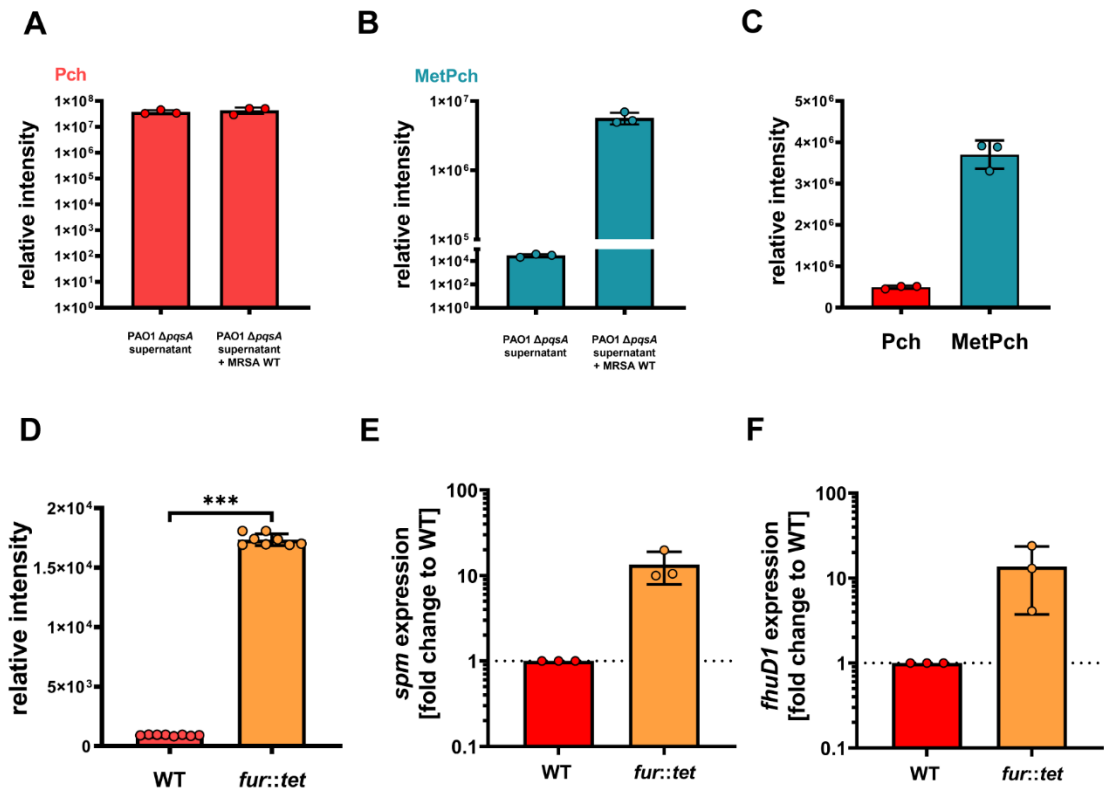

Figure S2: Metabolite quantification and *spm* regulation. Related to Figure 3. Quantification of **A)** Pch and **B)** MetPch in PAO1 Δ*pqsA* cell free SN after incubation with TSB (PAO1 Δ*pqsA* supernatant) or MRSA overnight culture (PAO1 Δ*pqsA* supernatant + MRSA WT) for 8h. **C)** Equimolar amounts of synthetic Pch and MetPch quantified by LC-MS. MetPch shows a 10-fold higher signal intensity than Pch, most likely due to more efficient ionization. **D)** The FhuD1/Smt promoter activity is elevated in a *fur* mutant (*fur*::*tet*) compared to its parent strain (WT). Expression of **E)** *spm* and **F)** *fhuD1* are elevated in a *fur* mutant (*fur*::*tet*) compared to its parent strain (WT). **A – C, E, F)** n = 3 biological replicates **D)** n = 8 biological replicates. Values are mean ± SDs ***$P \leq 0.001$ [Mann Whitney U test].

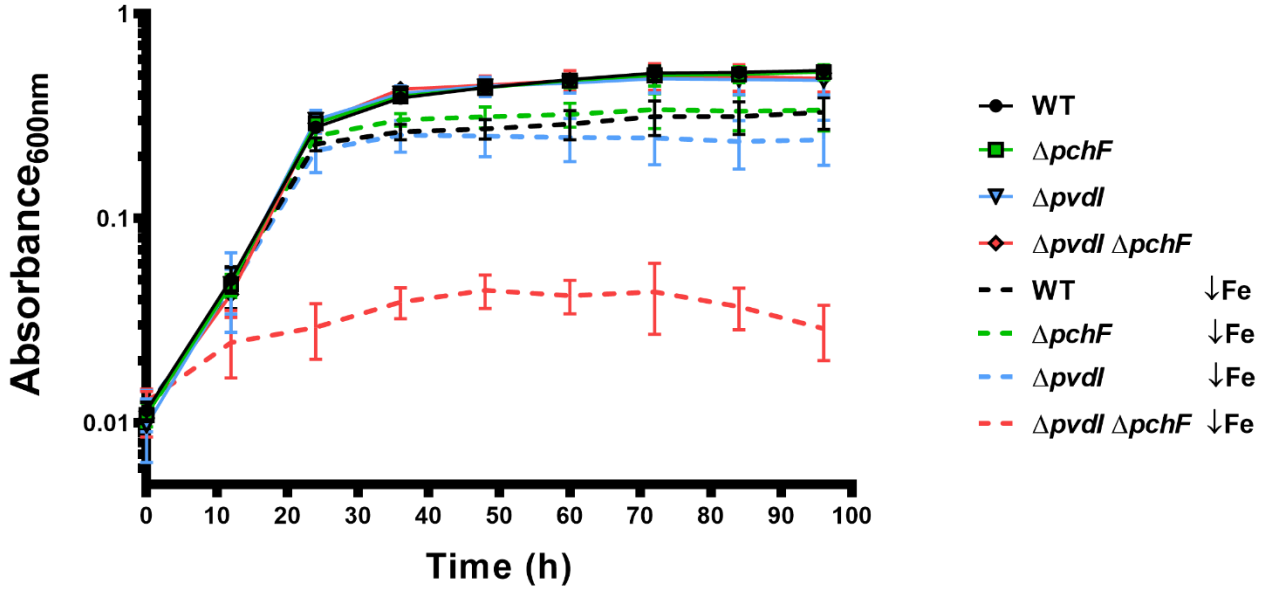

Figure S3: Siderophore biosynthesis is essential for growth under iron limited conditions. Related to Figure 3. 96-hour growth curves of *P. aeruginosa* PAO1 wild type (WT), a pyochelin mutant (Δ*pchF*), a pyoverdine mutant (Δ*pvdI*) and a mutant deficient in production of both siderophores (Δ*pchF* Δ*pvdI*). Cultures were grown in M9 minimal medium with glycerol as carbon source. To create a low iron environment (↓Fe), 500 µM 2,2'-Bipyridine was added to the growth medium. Growth curves of bacteria grown in standard M9 medium are shown as full lines and growth curves from low iron environment are shown as dashed lines. n = 5 biological replicates from two independent experiments.

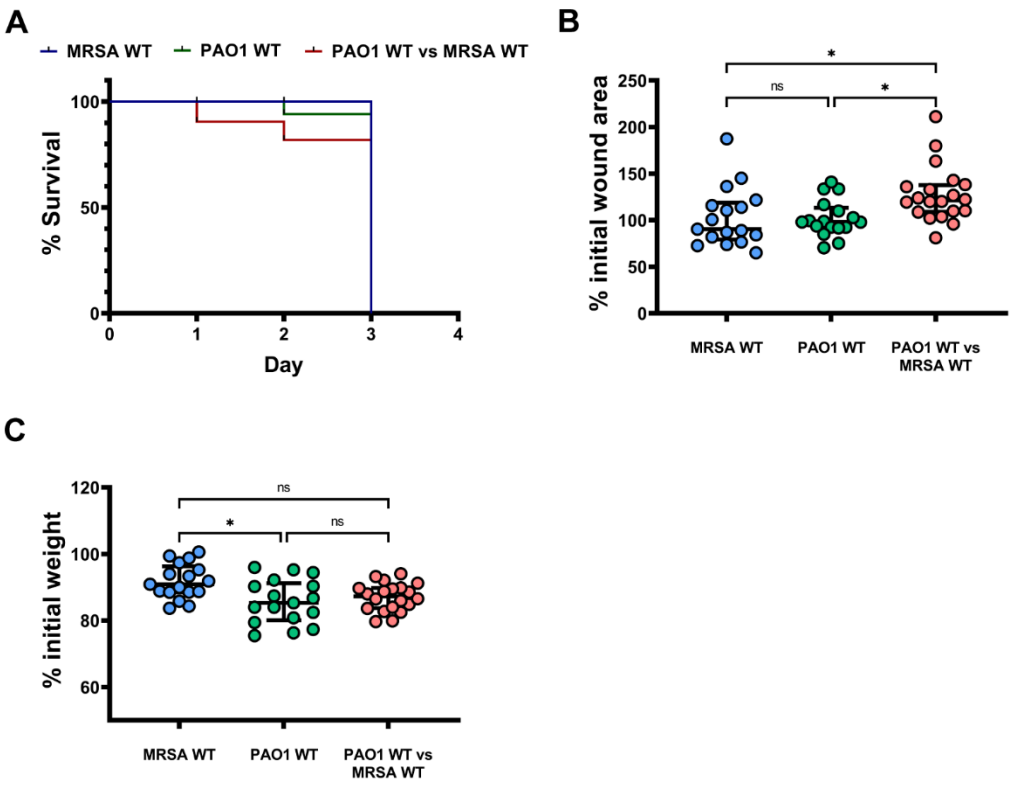

Figure S4: Polymicrobial infection leads to increased mortality and wound size progression. Related to Figures 4 and 5. **A**). Murine survival after skin wound infection with either *S. aureus* LAC wild type (MRSA WT) or *P. aeruginosa* PAO1 wild type (PAO1 WT) or co-infection (PAO1 WT vs MRSA WT). Co-infections lead to lowered murine survival compared with single infections. **B**) The change in murine skin wound size area between start and end of the experiment. Changes were calculated from the measured wound size area before bacterial infection and at the endpoint of the murine skin wound model. The changes are expressed as percent (%) of the initial wound size area at the endpoint of the experiment. **C**) The change in murine weight between start and end of the experiment. Changes were calculated from the murine weight before bacterial infection and at the endpoint of the murine skin wound model. The changes are expressed as percent (%) of the initial murine weight at the endpoint of the experiment. **A)** n = 17

biological replicates from 3 independent experiments. **B - C**) n = at least 17 biological replicates from 3 independent experiments. [Kruskal-Wallis with Dunn's multiple comparison]. Values are median ± interquartile range. Each dot represents values from a single mouse. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$, ns = not significant.

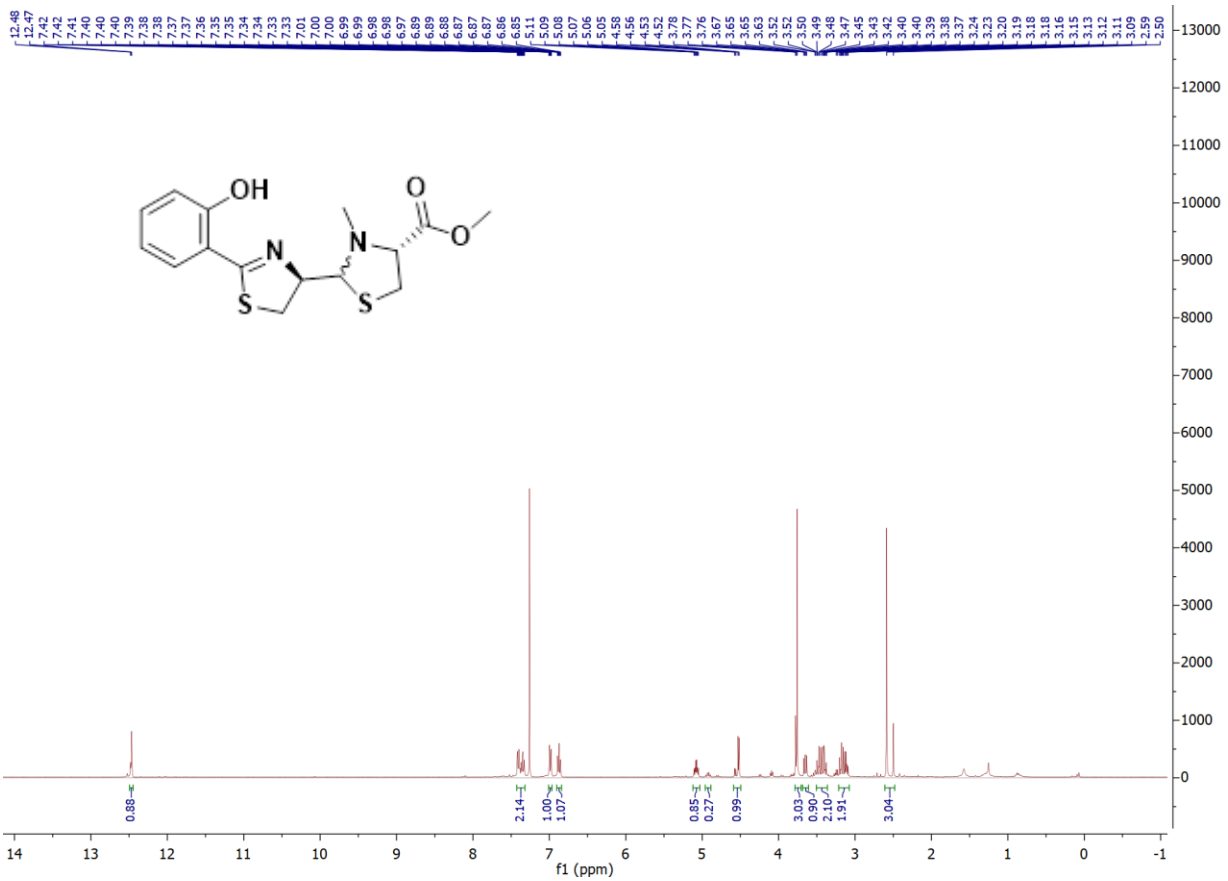

Figure S5: ^{1}H NMR of MetPch (400 MHz, Chloroform-*d*). Related to STAR Methods. [Major Isomer] δ 12.47 (s, 1H), 7.43 – 7.32 (m, 2H), 6.99 (ddd, *J* = 8.3, 3.4, 1.2 Hz, 1H), 6.87 (td, *J* = 7.6, 1.2 Hz, 1H), 5.08 (td, *J* = 8.8, 5.1 Hz, 1H), 4.52 (d, *J* = 5.2 Hz, 1H), 3.76 (s, 3H), 3.65 (dd, *J* = 9.2, 6.3 Hz, 1H), 3.51 – 3.36 (m, 2H), 3.22 – 3.08 (m, 2H), 2.59 (s, 3H).

Table S1: Primer used in this study. Related to STAR Methods.

primer name	primer sequence
construction primer	
spm_up_fw_KpnI	ccccggtacCGTGTTCAGCATCTGTCGAT
spm_up_rv_NcoI	ggggccatggTTTTGCTCGAGCTATCAAAGG
spm_dw_fw_NcoI	ggggccatggGTTCCAAAGAGACAGAGACATTAAG
spm_dw_rv_SalI	ccccgtcgacGGCTACTGCTGGCGAAGA
fhuD1_up_fw_KpnI	ccccggtaccAGAGGGGCACCACCAATAA
fhuD1_up_rv_NcoI	ggggccatggATCGACAGATGCTGAACACG
fhuD1_dw_fw_NcoI	ggggccatggGAAGATTATTGGTTCACAGATCCT
fhuD1_dw_rv_SalI	ccccgtcgacTCCCTAGAAAACAGCAGCTCA
fhuD2_up_fw_EcoRI	ccccgaattcAGTAAAGCGCCAAATGGTTG
fhuD2_up_rv_KpnI	ccccggtaccTTTCATAATTTCCTCCTATTGAAAATG
fhuD2_dw_fw_KpnI	ccccggtaccGCTGGTACATACTGGTACAACGA
fhuD2_up_fw_KpnI_new	ccccggtaccAGTAAAGCGCCAAATGGTTG
fhuG_up_fw_EcoRI	ccccgaattcTTTGCTGGATTTTTAGGTGCT
fhuG_up_rv_NcoI	ggggccatggTGCTATCAATTGTCTGCGTTT
fhuG_dw_fw_NcoI	ggggccatggATTGGTGCACCGTATTTCTT
fhuG_dw_rv_SalI	ccccgtcgaCCTTCCATTTCTGCAAGCTC
pchF_up_fw_XbaI	cccctctagaCGCGCCTGCTCGAAG
pchF_up_rv_HindIII	ccccaagcttCTCGCTCCAGAGTTCGATG
pchF_dwn_fw_HindIII	ccccaagcttCTGGCGCAATCCTTGTC
pchF_dwn_rv_EcoRI	ccccgaattCTCCAGCACCTGTTCCAG
pch_up_fw_EcoRI	ccccgaattCACTGCCTTCAACCTGCTC
pch_up_rv_NcoI	ggggccatggGTCGCCTGAAAACGAAGAC
pch_dwn_fw_NcoI	ggggccatggGCGGGCGAGGAAAGTT
pch_dwn_rv_XbaI	cccctctagaCGGTAGGTGTCCCACTTGA
pvdI_up_fw_XbaI	ggggtctaGATGCCGTCGTTGGTCAG
pvdI_up_rv_NcoI	ggggccatggACGCTTCTCAACGGGTAGTT
pvdI_dw_fw_NcoI	ggggccatggAGCGAACTAGAGGCGATCTG
pvdI_dw_rv_EcoRI	ccccgaattcTGAACATGGTCACACCCTTG
colony PCR primer	
fhuD2_chrom_col_fw	TGCCGATAATTGGGACGTAT
fhuD2_chrom_col_rv	GCATGCATACGCCATTCTTT
fhuG_chrom_col_fw	TTCAGGTGCTTCATTTGCTTT
fhuG_chrom_col_rv	ACGCTTCTACACGCGATTTT

spm_chrom_col	CCCCCTTTTCAACTGACAGA
fhuD1_chrom_col	CAAAAATAAACGCAACTGCAA
pch_chrom_col_up	CTGATCCTCCACGCCATC
pch_chrom_col_dwn	ATCGGCTTCCTGGTATTCG
pchF_chrom_col_up	CTCGACGAGGCGTTGC
pchF_chrom_col_dwn	CGATAGGTGCGCAGCAG
pvdI_chrom_col_up	TTGCCGGTATAAGGGTTCAG
pvdI_chrom_col_dwn	GCCTGGTGATTGAACATGAC
HC479	AGAAAAGCTTGCATTTTATTGAGAA
HC480	TTCAATAATTGTTTCCATAACCACA
mutant complementation primer	
spm_KI_up_fw_short_KpnI	ccccggtaccGGATTGAAGAGTGGGACGAT
spm_KI_up_rv_NcoI_NcoI_SalI	cccgtcgacatatccatggTCCCTAGAAAACAGCAGCTCA
spm_KI_dw_fw_NcoI	ggggccatggTGTCCCATTCCTTTTACGAGA
spm_KI_dw_rv_short_SalI	ccccgtcgaCAAGCAATCACCGACCTGTA
sequencing primer	
fhuD2_dw_seq	GTGCCGTTGTTATCGTTCAAT
fhuD2_up_seq	TGCTGCATCTTCCATAGGTG
fhuG_dw_seq	ACTCTGGGTGCGCAATTAAC
spm_seq1	CATCAAAATACATCAATACACCTTCA
spm_seq2	CCAGAATCAATAGAAATACGAAAAA
pchF_seq_up	CGGATCGCCCTGGTC
pchF_seq_dwn	GTCCAGGGTGGCGAAAC
pchcl_seq_up	ACAGGAGCGCACCGAAT
pchcl_seq_dwn	TCGTCGAGGCTGAACAGATT
pvdI_up_seq1	GACGATCAGGACGAAGAACC
pvdI_dwn_seq1	CAATCGCTGTTCGTCGAGT
plasmid primer	
pGPI_F	AGCTGATCCGGTGGATGAC
pGPI_R	ACGGTTGTGGACAACAAGC
pRT-PCR primer	
KK23 (gyrB_fw)	AACGGACGTGGTATCCCAGTTGAT
KK24 (gyrB_rv)	CCGCCAAATTTACCACCAGCATGT
KK55 (spm_fw)	GTGTTACTAAGAGGCTTATCAAGGA
KK56 (spm_rv)	ACCTTGTATCTAAGCCACAACC

KK63 (fhuD1_fw)	GGTCAAGCAACAGCATCTG
KK64 (fhuD1_rv)	GAACAATATCCAAACCACGACC

Restriction sites are underlined

Table S2: Solvents used for solvent screen. Related to STAR Methods.

solvent 1	dimethyl formamide
solvent 2	dichloromethane
solvent 3	ethyl acetate
solvent 4	ethyl acetate + 0.1 % formic acid
solvent 5	methanol
solvent 6	water
solvent 7	acetonitrile + water (1:1)
solvent 8	acetonitrile + water (1:1) + 0.1% formic acid
solvent 9	dimethyl formamide + water
solvent 10	dimethyl formamide + water + 0.1 % formic acid
solvent 11	Methanol + water (1:1) + 0.1% formic acid
solvent 12	ethyl acetate + methanol (1:1)
solvent 13	ethyl acetate + methanol (1:1) + 0.1 % formic acid
solvent 14	n-Butanol
solvent 15	n-Butanol + 0.1% formic acid