Table S 1: Bacterial strains used in this study

Bacterial Strains	Genotype	Reference
***E. coli* S17-1**	Conjugation strain*; recA pro hsdR*; RP42Tc::Mu-Km::Tn7 integrated into the chromosome	ATCC 47055
***P. polymyxa* DSM 365**	wild type	DSMZ
P. polymyxa* DSM 365 *Δldh1	DSM 365 Δ*ldh1*	Schilling et al (2020)
P. polymyxa* DSM 365 *ΔpepCQ	DSM 365 *ΔpepC*	This study
P. polymyxa* DSM 365 *ΔpepQ	DSM 365 *ΔpepQ*	This study

In addition to the strains listed above, each plasmid listed in Table S 2 was used for the transformation of *E. coli* S17-1 as well as for the conjugational transformation of *P. polymyxa* DSM 365.

Table S 2: Plasmids used in this study

Plasmids	Description	Reference
pCasPP	*P. polymyxa* CRISPR-Cas9 genome editing plasmid	Rütering et al (2017)
pCasPPH_pepC	*pepC* targeting knock out plasmid containing repair template	This study
pCasPPH_pepQ	*pepQ* targeting knock out plasmid containing repair template	This study
pCRai	PsgsE-*AsdCas12a*; Pgapdh-off target gRNA; *neo*; oriT, *repU*	This study
pCRaiGFP	PsgsE-*AsdCas12a*; Pgapdh-off target gRNA;*neo*; oriT, *repU*; PsgsE-*sfGFP*	This study
pCRaiGmR_soxS	PsgsE-*AsdCas12a-soxS*; Pgapdh-off target gRNA; *neo*; *oriT*, *repU*; PsgsE_*mRFP*-US-PsgsE-*sfGFP*	This study
pCRaiGFP_GltC	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - off target gRNA	This study
pCRaiGFP_GltC_a1	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a1	This study
pCRaiGFP_GltC_a2	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a2	This study
pCRaiGFP_GltC_a3	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a3	This study
pCRaiGFP_GltC_a4	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a4	This study
pCRaiGFP_sig70	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - off target gRNA	This study
pCRaiGFP_sig70_a1	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a1	This study
pCRaiGFP_sig70_a2	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a2	This study
pCRaiGFP_sig70_a3	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a3	This study
pCRaiGFP_sig70_a4	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a4	This study
pCRai_GFP_rpoD_a1	Test plasmid for CRISPRa targeting US region of PsgsE-	This study

	sfGFP - sgRNA_a1	
pCRai_GFP_rpoD_a2	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a2	This study
pCRai_GFP_rpoD_a3	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a3	This study
pCRai_GFP_rpoD_a4	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a4	This study
pCRai_GFP_rpoD	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - off target gRNA	This study
pCRai_GFP_soxS_a1	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a1	This study
pCRai_GFP_soxS_a2	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a2	This study
pCRai_GFP_soxS_a3	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a3	This study
pCRai_GFP_soxS_a4	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a4	This study
pCRai_GFP_soxS	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - off target gRNA	This study
pCRai_GFP_CRP_a1	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a1	This study
pCRai_GFP_CRP_a2	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a2	This study
pCRai_GFP_CRP_a3	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a3	This study
pCRai_GFP_CRP_a4	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - sgRNA_a4	This study
pCRai_GFP_CRP	Test plasmid for CRISPRa targeting US region of PsgsE-*sfGFP* - off target gRNA	This study
pCRaiGFP_soxS_sfGFP_T1	Test plasmid for *sfGFP* repression sgRNA_T1	This study
pCRaiGFP_soxS_sfGFP_T2	Test plasmid for *sfGFP* repression sgRNA_T2	This study
pCRaiGFP_soxS_sfGFP_T3	Test plasmid for sfGFP repression sgRNA_T3	This study
pCRai_pepQ_T1	Plasmid for repression of *pepQ* sgRNA T1	This study
pCRai_pepCQ_T1	Plasmid for dual repression of *pepQ* and *pepC*	This study
pCRai_soxS_ldhmultibdh1	Multiplex knock-down of repression of *ldh2*, *ldh3*, *ldh4* and activation of P*bdh* (sgRNA 1)	This study
pCRai_soxS_ldhmultibdh2	Multiplex knock-down of repression of ldh2, ldh3, ldh4 and activation of Pbdh (sgRNA 2)	This study
pCRai_soxS_ldhmultibdh3	Multiplex knock-down of repression of ldh2, ldh3, ldh4 and activation of Pbdh (sgRNA 3)	This study
pCRaiGmR_soxS_mT2	PsgsE-mRFP-US-PsgsE_sfGFP expression plasmid with gRNA for mRFP repression (sgRNA T2)	This study
pCRaiGmR_soxS_dualT2	PsgsE-*mRFP*-US-PsgsE_*sfGFP* expression plasmid with gRNA for *mRFP* repression (sgRNA T2) and GFP activation (sgRNA a1)	This study
pCRaiGmR_soxS_GFPa1	PsgsE-*mRFP*-US-PsgsE_*sfGFP* expression plasmid with gRNA and GFP activation (sgRNA a1)	This study

Table S 3: Oligonucleotides and primers used in this study. Overhangs used for Golden Gate Assembly or Gibson isothermal assembly are depicted in lower case. Restriction sites are underlined.

Name	SEQUENCE 5'->3'	Comment
soxS_fw	ATGTTGGTCTCAACGCTGATGTCCCATCAGAAAATTATTCAGGATC	Cloning of activator domain
soxS_rev	TAAAAGGTCTCGTACCATTTACAGGCGGTGGCGATAATCG	Cloning of activator domain
sig70_fw	ATGTTGGTCTCAACGCTGATGATAGAGGACACACCATTCCGT	Cloning of activator domain
sig70_rev	TAAAAGGTCTCGTACCATTCATTGCCATTCCTCCTCCTTCC	Cloning of activator domain
cAMP_fw	ATGTTGGTCTCAACGCTGATGCATCCCTATTATCGTTCATTAGAAAAAA	Cloning of activator domain
cAMP_rev	TAAAAGGTCTCGTACCATTTAATGCGGATCACTACGCAGCAA	Cloning of activator domain
GltC_fw	ATGTTGGTCTCAACGCTGGTGGAATTACGACAGTTACAATACTTCATGAA	Cloning of activator domain
GltC_rev	TAAAAGGTCTCGTACCATTCACTGCCCTAAATCCGCTTTTG	Cloning of activator domain
RpoD_fw	ATGTTGGTCTCAACGCTGGTGCTGTATCCTTCCATTGATGAAATG	Cloning of activator domain
RpoD_rev	TAAAAGGTCTCGTACCATTTATTCTTCTTCGCCGCTTTCAAGT	Cloning of activator domain
As_CRISPR_fw	GGGGATACGCTAATTTCTACTCTTGTAGATAAGTCTTCTCAGCCG	Construction of pCRai
As_CRISPR_rev	AAATCCAGATGGAGTATGTCTTCACCGGTGGAAAGCG	Construction of pCRai
AsCRISPR_vec_fw	CACCGGTGAAGACATACTCCATCTGGATTTGTTCAGAACGC	Construction of pCRai
AsCRISPR_vec_rev	GTAGAAATTAGCGTATCCCCTTTCAGATACTCGCAC	Construction of pCRai
enAsCPF1_fw	TTAGGCTTTTACTTAATGACACAGTTTGAAGGCTTTACGAATCTG	Construction of pCRai
enAsCPF1_rev	CGTAGATCTGAATTCTTATTACCCGAGACCTACCCAATGCG	Construction of pCRai
enAsCPF1_Vector.fw	GGTCTCGGGTAATAAGAATTCAGATCTACGCGTTCCCCGC	Construction of pCRai
enAsCPF1_Vector.REV	TTCAAACTGTGTCATTAAGTAAAAGCCTAAAATCCCCCTTCGTT	Construction of pCRai
GFP_vecint_rev	gcaacgcggccttttacggTTCCTGGCCATATGACGATCCTCCTTACCTCTCATTG	*sfGFP* plasmid cloning
GFP_vecint_fw	gcccgcaaccggcgcatcaagcccgccGACTAGCAATATGAAACACGGAAAAAATCAAGC	*sfGFP* plasmid cloning
sgsE-a1_fw	agatTTTATCTCACATAATAGGGCT	Test gRNA for activation of PsgsE-*sfGFP*
sgsE-a1_rev	gagtAGCCCTATTATGTGagatAA	Test gRNA for activation of PsgsE-*sfGFP*
sgsE-a2_fw	agatAGTCTATATCAATCGGTAAC	Test gRNA for activation of PsgsE-*sfGFP*
sgsE-a2_rev	gagtGTTACCGATTGATATAGACT	Test gRNA for activation of PsgsE-*sfGFP*

sgsE-a3_fw	agatATCCTCATATTTTCCTAGTA	Test gRNA for activation of PsgsE-*sfGFP*
sgsE-a3_rev	gagtTACTAGGAAAATATGAGGAT	Test gRNA for activation of PsgsE-*sfGFP*
sgsE-a4_fw	agatCATCGATGGCGACATTGATA	Test gRNA for activation of PsgsE-*sfGFP*
sgsE-a4_rev	gagtTATCAATGTCGCCATCGATG	Test gRNA for activation of PsgsE-*sfGFP*
sfGFP1_T1_fw	agatCGTGCGTGGCGAGGGTGAAG	Test gRNA for repression of PsgsE-*sfGFP*
sfGFP1_T1_rev	gagtCTTCACCCTCGCCACGCACG	Test gRNA for repression of PsgsE-*sfGFP*
sfGFP_T2_fw	agatCCATTAGTTGCGTCACCTTC	Test gRNA for repression of PsgsE-*sfGFP*
sfGFP_T2_rev	gagtGAAGGTGACGCAACTAATGG	Test gRNA for repression of PsgsE-*sfGFP*
sfGFP_T3_fw	agatAGCTCAATGCGGTTTACCAG	Test gRNA for repression of PsgsE-*sfGFP*
sfGFP_T3_rev	gagtCTGGTAAACCGCATTGAGCT	Test gRNA for repression of PsgsE-*sfGFP*
mRFP_T_fw	agatAAAGTTCGTATGGAAGGTTC	gRNA for repression of PsgsE-*mRFP*
mRFP_T_rev	gagtGAACCTTCCATACGAACTTT	gRNA for repression of PsgsE-*mRFP*
sfGFP_dual_fw	agatTTATCTCACATAATAGGGCTTAATTTCTACTC	Test gRNA for repression of PsgsE-*mRFP* and simultaneous activation of PsgsE-*sfGFP*
sfGFP_dual_rev	acaaGAGTAGAAATTAAGCCCTATTATGTGAGATAA	Test gRNA for repression of PsgsE-*mRFP* and simultaneous activation of PsgsE-*sfGFP*
mRFP_dual_fw	ttgtAGATAAAGTTCGTATGGAAGGTTC	Test gRNA for repression of PsgsE-*mRFP* and simultaneous activation of PsgsE-*sfGFP*
mRFP_dual_rev	gagtGAACCTTCCATACGAACTTTATCT	Test gRNA for repression of PsgsE-*mRFP* and simultaneous activation of PsgsE-*sfGFP*
GGA_sfGFP_fw	AATTGGTCTCAAACTCAATATGAAACACGGAAAAAATCAAGCAG	Cloning of PsgsE-*mRFP*-US-PsgsE-*sfGFP* expression cassette
GGA_sfGFP_rev	AATTGGTCTCACTAGTATGACGATCCTCCTTACCTCTCATTG	Cloning of PsgsE-*mRFP*-US-PsgsE-*sfGFP* expression cassette
GGA_mRFP_fw	AATTGGTCTCATCGACCTGCATACTAGCCTGTTACAGGCATATTCATATCAATGTC	Cloning of PsgsE-*mRFP*-US-PsgsE-*sfGFP* expression cassette
GGA_mRFP_rev	CTTTGGTCTCAAGTTAAGCACCGGTGgagtGACGAC	Cloning of PsgsE-*mRFP*-US-PsgsE-*sfGFP* expression cassette

pepQ_T1 fw	agatCACCAGCAGTGCAGACAATC	gRNA for repression of *pepQ*
pepQ_T1_rev	gagtGATTGTCTGCACTGCTGGTG	gRNA for repression of *pepQ*
pepCQ_T1_fw	agatCCTGACATGACAGCCTCGCCTAATTTCTACT	gRNAs for dual repression of *pepCQ*
pepCQ_T1_rev	caagAGTAGAAATTAGGCGAGGCTGTCATGTCAGG	gRNAs for dual repression of *pepCQ*
pepCQ2_T1_fw	cttgTAGATCACCAGCAGTGCAGACAATC	gRNAs for dual repression of *pepCQ*
pepCQ_T1_rev	gagtGATTGTCTGCACTGCTGGTGATCTA	gRNAs for dual repression of *pepCQ*
bdh1_fw	cttgTAGATGACACTCATTCTGTGGTATA	gRNA cloning for *Ppbdh* activation
bdh1_rev	gagtTATACCACAGAATGAGTGTCATCTA	gRNA cloning for *Ppbdh* activation
bdh2_fw	cttgTAGATTTCTTGTCTTTGCTTCAATT	gRNA cloning for *Ppbdh* activation
bdh2_rev	gagtAATTGAAGCAAAGACAAGAAATCTA	gRNA cloning for *Ppbdh* activation
bdh3_fw	gagtGCTCGTTACTTTTATACAAA	gRNA cloning for *Ppbdh* activation
bdh3_rev	gagtTTTGTATAAAAGTAACGAGCATCTA	gRNA cloning for *Ppbdh* activation
sfGFP_qPCR_fw	CCCTATTCTGGTGGAACTGGATGG	qPCR primer
sfGFP_qPCR_rev	CAGTAGTACAGATGAACTTCAGCGTC	qPCR primer
gyrA_qPCR_fw	GAGATATGGCCGCTGCGATG	qPCR primer
gyrA_qPCR_rev	GCTCTCTTCACCATCGTAGTTCGG	qPCR primer
mRFP_qPCR_fw	TACCTGAAACTGTCCTTCCCGG	qPCR primer
mRFP_qPCR_rev	GTAGATGAACTCACCGTCTTGCAGG	qPCR primer
ldh3_qPCR_fw	GAGTCATTGGATCA GA CGTTGC	qPCR primer
ldh3_qPCR_rev	AACTCGGAGTCACCGTGTTCTCC	qPCR primer
ldh2_qPCR_fw	GATTATCGGGGTTGGACGCATTGG	qPCR primer
ldh2_qPCR_rev	CAAGGTTGTGAATGGAATGACTTCCTTG	qPCR primer
ldh4_qPCR_fw	GAATGAGCCGGACCTGGATGCG	qPCR primer
ldh4_qPCR_rev	CAATCCTCGACTGGATCGGTGTTC	qPCR primer
bdh_qPCR_fw	AGATGCTCAGCATCCATTGACTGG	qPCR primer
bdh_qPCR_rev	CAACGACACGGTCGCCAACCTG	qPCR primer

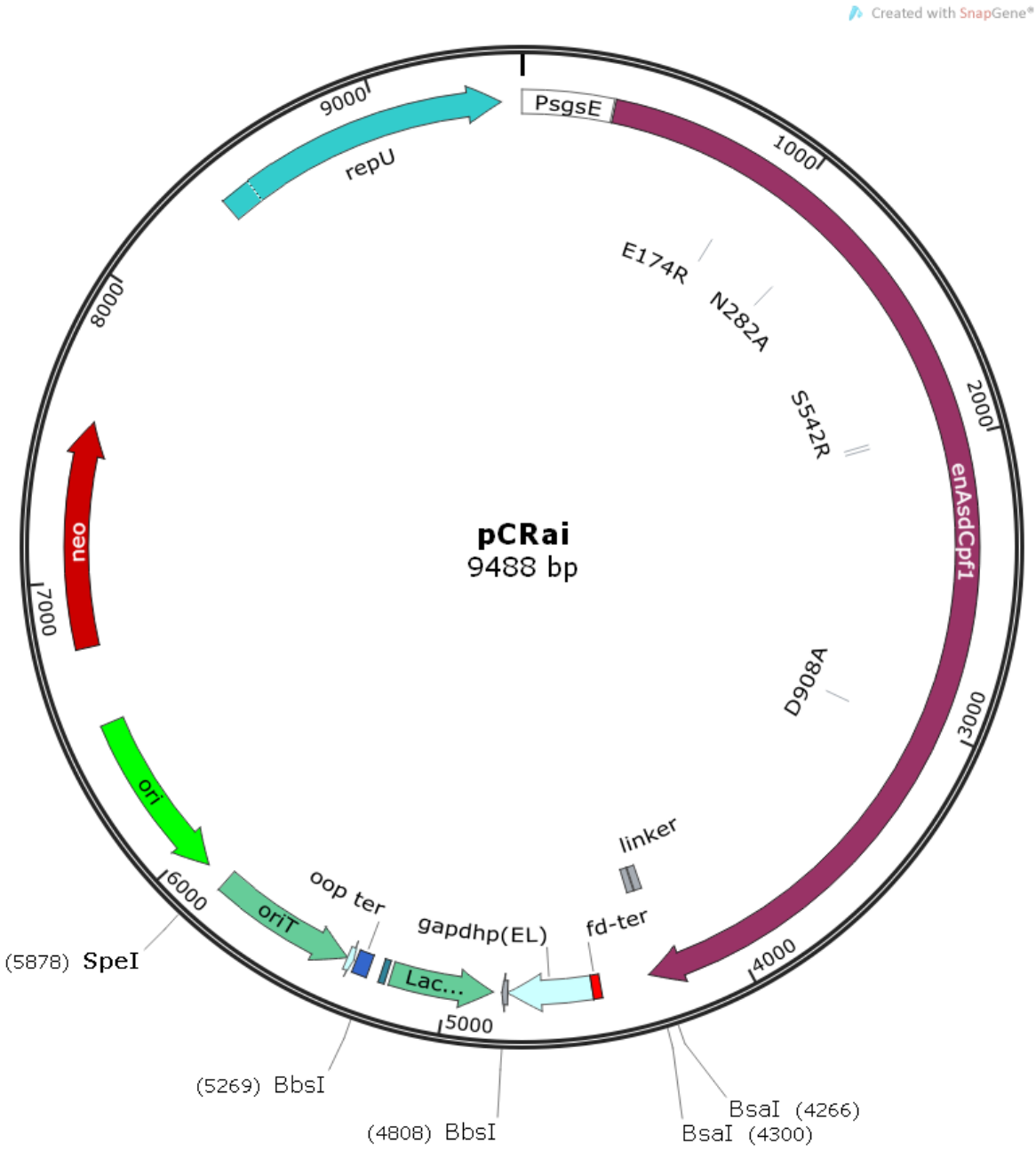

Figure S 1: Plasmid map of pCRai. The conjugational plasmid based on the pUB110 and pCasPP[2] plasmids was constructed via Gibson Assembly by replacing the Cas9-gRNA expression cassette with an engineered catalytically inactive variant of Cas12a[3] (E174R, N282A, S542R, K548R, D908A) and an appropriate CRISPR-array. Variable activator domains can be cloned by replacing a BsaI-cassette linked via a 10 aa flexible linker. The gRNAs can be cloned by Golden Gate Assembly using BbsI by replacing the *lacZ* expression cassette.

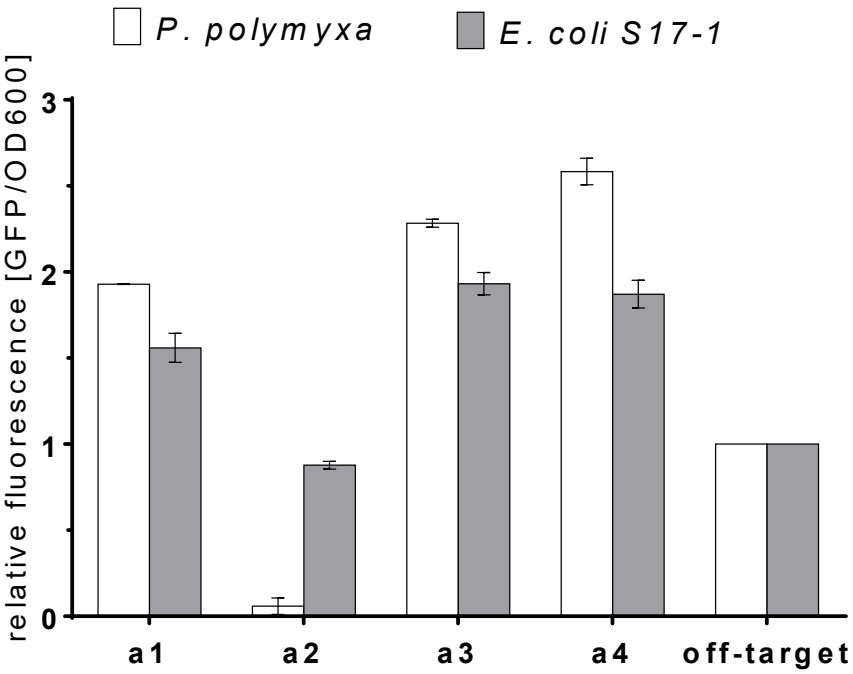

Figure S 2: Relative fluorescence levels of *P. polymyxa* and *E. coli* S17-1 harboring pCRaiGFP_soxS variants. Four different gRNAs (a1-a4) were expressed and normalized fluorescence levels (Ex. 488 nm Em. 515 nm) were compared to a strain expressing an off-target gRNA. The gRNAs a1, a3 and a4 showed increased GFP fluorescence in *P. polymyxa* as well as *E. coli* S17-1 demonstrating similar effects in both, Gram-positive and Gram-negative host organisms.

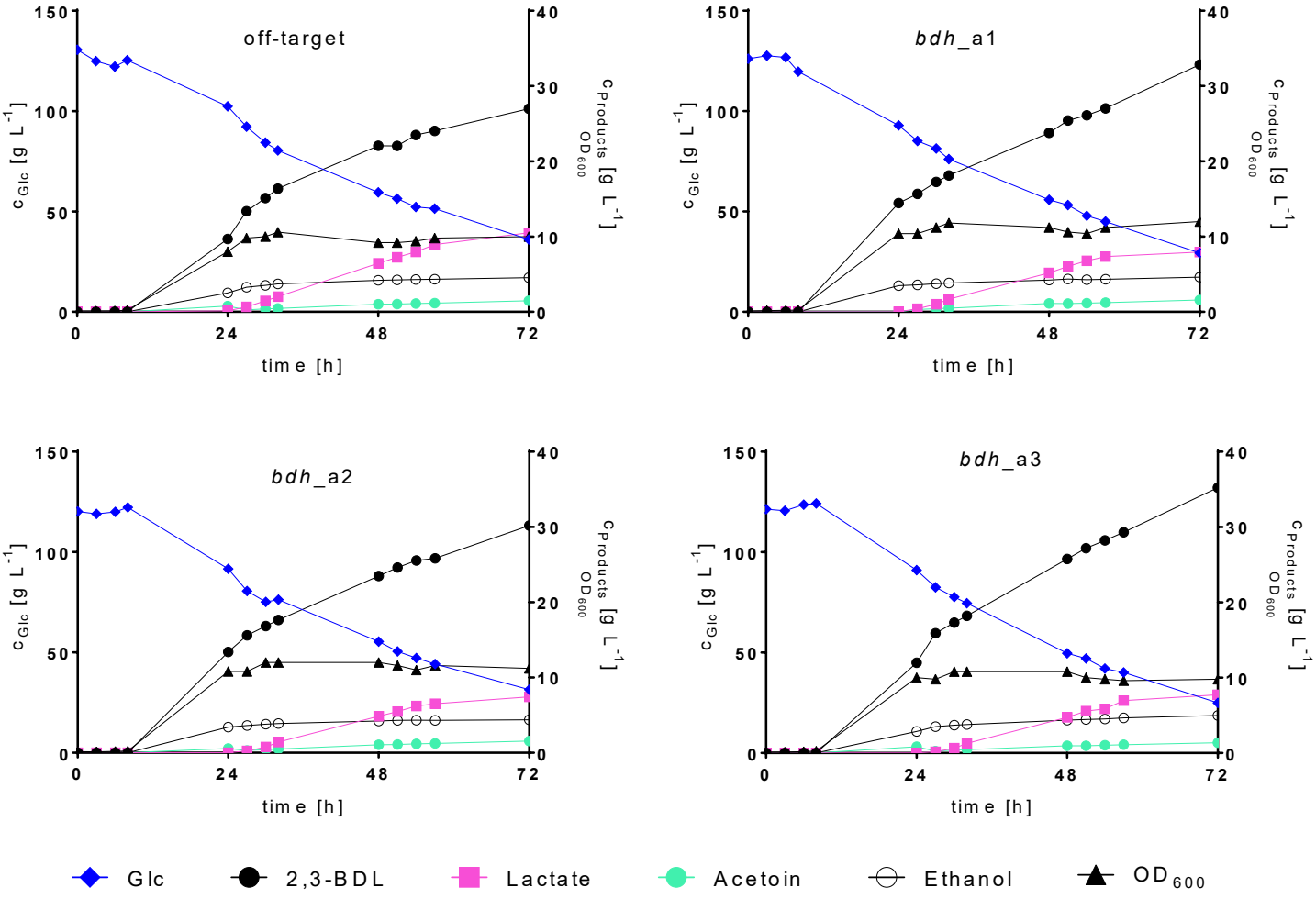

Figure S 3: Profiles of 2,3-BDL fermentations. Overview of fermentation profiles of single batch fermentations. *P. polymyxa* DSM 365 was transformed with pCRai_soxS encoding either off-target gRNAs or a multiplex CRISPR-array targeting the open reading frame of three lactate dehydrogenases and the upstream region of P_{bdh} with three gRNAs (bdh_a1-a3) individually. Lactate production was reduced by ~ 20 % in all variants expressing the corresponding gRNAs. Glc: Glucose; 2,3-BDL: 2,3-*R,R*-butantediol

Table S 4 Final product titers and 2,3-BDL yield (g_{BDL} g_{Glc}^{-1}) of 2,3-BDL fermentations. *P. polymyxa* DSM 365 was transformed with pCRai_soxS encoding either off-target gRNAs or a multiplex CRISPR-array targeting the open reading frame of three lactate dehydrogenases and the upstream region of P_{bdh} with three gRNAs (bdh_a1-a3) individually**.** Values represent mean and deviation of biological duplicates after 72 h cultivation at microaerobic conditions.

	2,3-BDL	Ethanol	Acetoin	Lactate	Formate	Y_{PS}
off-target	27.46 ± 0.47	4.21 ± 0.33	1.34 ± 0.14	10.30 ± 0.20	0.34 ± 0.34	0.29 ± 0.01
***bdh*_a1**	34.30 ± 1.45	4.84 ± 0.23	1.50 ± 0.08	7.69 ± 0.26	0.72 ± 0.03	0.34 ± 0.00
***bdh*_a2**	32.44 ± 2.26	4.64 ± 0.25	1.42 ± 0.15	7.74 ± 0.30	0.72 ± 0.04	0.34 ± 0.00
***bdh*_a3**	34.74 ± 0.50	4.89 ± 0.10	1.22 ± 0.08	7.81 ± 0.08	0.67 ± 0.07	0.35 ± 0.02

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