

Supplementary information, Table S3 Strains, plasmids and primers used in this study

Strain, plasmid or primers	Relevant phenotype or description	Source or reference
<i>Strains</i>		
PA14	Wild type	Our lab
ΔTCR	PA14, total <i>crispr</i> and <i>cas</i> region deleting	2
Δ <i>csy1</i>	PA14, <i>csy1</i> deleting	2
Δ <i>cas1</i>	PA14, <i>cas1</i> deleting	2
Δ <i>csy2</i>	PA14, <i>csy2</i> deleting	2
Δ <i>csy3</i>	PA14, <i>csy3</i> deleting	2
Δ <i>cas3</i>	PA14, <i>cas3</i> deleting	2
Δ <i>csy4</i>	PA14, <i>csy4</i> deleting	2
ΔCR1	PA14, <i>crispr</i> region 1 deleting	2
ΔCR2	PA14, <i>crispr</i> region 2 deleting	2
Δ <i>lasR</i>	PA14, <i>lasR</i> deleting	3
ΔTCR Δ <i>lasR</i>	PA14, <i>lasR</i> and total <i>crispr</i> and <i>cas</i> region double deleting	This study
ΔTCR/p-TCR	ΔTCR, pAK1900-TCR	This study
Δ <i>lasR</i> /p- <i>lasR</i>	Δ <i>lasR</i> , pAK1900- <i>lasR</i>	This study
Δ <i>csy1</i> /p- <i>csy1</i>	PA14, <i>csy1</i> deleting, pMQ70- <i>csy1</i>	2
Δ <i>csy2</i> /p- <i>csy2</i>	PA14, <i>csy2</i> deleting, pMQ70- <i>csy2</i>	2
Δ <i>csy3</i> /p- <i>csy3</i>	PA14, <i>csy3</i> deleting, pMQ70- <i>csy3</i>	2
Δ <i>csy4</i> /p- <i>csy4</i>	PA14, <i>csy4</i> deleting, pMQ70- <i>csy4</i>	2
Δ <i>cas3</i> /p- <i>cas3</i>	PA14, <i>cas3</i> deleting, pMQ70- <i>cas3</i> His	2
Δ <i>cas3</i> /p- D124A	PA14, <i>cas3</i> deleting, pMQ70- <i>cas3</i> (D124A)His	This study
Δ <i>cas3</i> /p-K427	PA14, <i>cas3</i> deleting, pMQ70- <i>cas3</i> (K427A)His	This study
Δ <i>cas3</i> /p-D576A	PA14, <i>cas3</i> deleting, pMQ70- <i>cas3</i> (D576A)His	This study
Δ <i>cas3</i> /p-P611A	PA14, <i>cas3</i> deleting, pMQ70- <i>cas3</i> (P611A)His	This study
Δ <i>cas3</i> /p-E825A	PA14, <i>cas3</i> deleting, pMQ70- <i>cas3</i> (E825A)His	This study
Δ <i>cas3</i> /p-R852A	PA14, <i>cas3</i> deleting, pMQ70- <i>cas3</i> (R852A)His	This study
ΔCR1/p-CR1	PA14, <i>crispr</i> region 1 deleting, pgRNA-CR1	This study
ΔCR1/p-crRNA1-12	PA14, <i>crispr1</i> deleting, pgRNA-crRNA1-12	This study
PA14-EGFP	PA14, pMQ70- <i>egfp</i>	This study
ΔTCR-EGFP	ΔTCR, pMQ70- <i>egfp</i>	This study
Δ <i>lasR</i> -EGFP	Δ <i>lasR</i> , pMQ70- <i>egfp</i>	This study
PAO1	Wild type	Our lab
KP NTUH-2044	Wild type	4
<i>E. coli</i> BL21 pCsy1-4	BL21, pHMGWA-Csy1-4	5
<i>E. coli</i> K12	Wild type	Our lab
<i>Plasmids</i>		
pMQ70	Arabinose-inducible expression vector; Cb ^r Ap ^r	6
pMQ70- <i>cas3</i> His	PA14- <i>cas3</i> complementation construct; Cb ^r Ap ^r	This study
pMQ70- <i>cas3</i> (D124A)His	PA14- <i>cas3</i> complementation construct with catalytic residue D124A mutant; Cb ^r Ap ^r	This study
pMQ70- <i>cas3</i> (K427A)His	PA14- <i>cas3</i> complementation construct with catalytic residue K427A mutant; Cb ^r Ap ^r	This study

pMQ70- <i>cas3</i> (D576A)His	PA14_ <i>cas3</i> complementation construct with catalytic residue D576A mutant; Cb ^r Ap ^r	This study
pMQ70- <i>cas3</i> (P611A)His	PA14_ <i>cas3</i> complementation construct with catalytic residue P611A mutant; Cb ^r Ap ^r	This study
pMQ70- <i>cas3</i> (E825A)His	PA14_ <i>cas3</i> complementation construct with catalytic residue E825A mutant; Cb ^r Ap ^r	This study
pMQ70- <i>cas3</i> (R852A)His	PA14_ <i>cas3</i> complementation construct with catalytic residue R852A mutant; Cb ^r Ap ^r	This study
pMQ70- <i>egfp</i>	Enhanced green fluorescent protein construct; Cb ^r Ap ^r	This study
pgRNA	Bacterial guide RNA expression vector	Our lab
pgRNA-CR1	PA14_CR1 complementation construct	This study
pgRNA-crRNA1-12	PA14_crRNA1-12 complementation construct	This study
pAK1900	<i>P. aeruginosa</i> expression vector; Ap ^r	Our lab
plasR	pAK1900-lasR; Ap ^r	This study
pTCR	pAK1900-TCR; Ap ^r	This study
pCVD442	<i>P. aeruginosa</i> gene knockout vector; Ap ^r	Our lab
pKO-lasR	pCVD442-lasR-flank	This study

Primers for qRT-PCR

Csy1 F	CCGCAGAACATCAGTCAGTT
Csy1 R	ATGCTCGAAGACCGAAGAGT
Cas1 F	GACATTTCTCCCAGCGAACT
Cas1 R	TGTTCCAGTAGTGCGAATGC
Csy2 F	AGTCGGAATCTCCCTCGATA
Csy2 R	TCAGGTTGAAGACCTTGGTG
Csy3 F	ATGTCCTGCTCGAAGTGGT
Csy3 R	CTTGCTCTTCTGGCCTTCT
Cas3 F	CGACAACCTCGATGAACTGCT
Cas3 R	GCGAGTACGACGAACAGATG
Csy4 F	CCGTACCGTCAGGTCAGTC
Csy4 R	GAGCCTCCTCCTCACTCAGA
CR1 F	TGCCGTATAGGCAGCTAAGA
CR1 R	ACGACCTATGTGGCAGTGAA
CR2 F	TGAACTTATCAAGCGGCTGT
CR2 R	CTCGAGCACGATTGTTCACT
16S F	TGGTTTAATTCGAAGCAACG
16S R	ATCTCACGACACGAGCTGAC
gacA F	CCGACTGCGGTGAAGACTGT
gacA R	GGTGACTACCACGACCTTGATG
rsmY F	GCCAAAGACAATACGGAAAC
rsmY R	TCTATCCTGACATCCGTGCT
rsmA F	TACTGGGTGTCAAAGGGAAC
rsmA R	ATGGTTTGGCTCTTGATCTT
lasR F	CTTCATCGTCGGCAACTAC
lasR F	GTCTGGTAGATGGACGGTTC

lasI F	TGCGTGCTCAAGTGTTCAAGG
lasI R	TGTCCAGAGTTGATGGCGAAA
rhIR F	GCTCCTCGGAAATGGTGGT
rhIR R	GGAAAGCACGCTGAGCAAAT
rhII F	TCCGCAAACCCGCTACATC
rhII R	TCTCGCCCTTGACCTTCTGC
rpoS F	GCCTGAACGAACGGGTGACT
rpoS R	CACCTCACGCTGCTTGTCG
algC F	GGAGAACCTGAAGGACCTGA
algC R	AAGCCGAGAACGCCGACCT
exsA F	GGTAAACAAGGAAGAGGGCGTAT
exsA R	GGACGAAGCCTTG TAGAACTGG
exoT F	CCGAGATCAAGCAGATGATG
exoT R	GTGATCCTTCGCCAGTCTCT
exoU F	TTGCCGAGAACATTGTAAG
exoU R	TTCAGCGCTTGATTGATTTC
exoY F	AATCGTCACTACGCTGATCG
exoY R	CTGGTCGACACAGATGAACC
bfmR F	GCGAGCTGGTAGGCAACTA
bfmR R	GATGTCGAGGACGATCAGG
bfmS F	GACTACCTCAAGGAGCGCAT
bfmS R	CTCTTCGAGATCGTTCCACA
Hu TNF F	TTTGATCCCTGACATCTGGA
Hu TNF R	GGCCTAAGGTCCACTTGGT
Hu IL1b F	TCAGCCAATCTTCATTGCTC
Hu IL1b R	TCAGCCAATCTTCATTGCTC
Hu IL6 F	GCTGCAGGACATGACAACCTC
Hu IL6 R	CTGCGCAGAATGAGATGAGT
Hu-Gapdh F	TGGTATCGTGGAAGGACTCA
Hu-Gapdh R	CCAGTAGAGGCAGGGATGAT
KP CR1 F	ACAGGATAGAGCCAAATCCG
KP CR1 R	ATCCTTCGCTATGTCTTCCC
KP CR2 F	CAGGCGTTATTGTCATCCAC
KP CR2 R	TTCGCAAGCACTACGTCTTC
KP 16S F	GTCGTAGTCCGGATTGGAGT
KP 16S R	GTATTCACCGTGGCATTCTG
EC CR1 F	CTGGCTGGCAATCTCTTTC
EC CR1 R	AAATCCGGAGATACGGAAACT
EC CR2 F	CCGTGTATCTGCTCGGTTTA
EC CR2 R	TTAACGCTCACCAGCATTTC
EC 16S F	GTAGCGGTGAAATGCGTAGA
EC 16S R	CAAATCGACATCGTTTACGG

Primers for plasmid construction

Cas3 F CGGAATTCATGAACATCCTGCTGGTGTCTG (*EcoR* I)

Cas3 C His R	CCCAAGCTTTCAATGATGATGATGATGATGGTTGTATTCTTGAAC CCC (<i>Hind</i> III)
D124A F	CTGTTCCACGGAATCGGCAAGGCCAGCCAG
D124A R	CTGGCTGGCCTTGCCGATTCCGTGGAACAG
K427A F	TCGACCGGTTGCGGTGGAACCCTCGCCAAC
K427A R	GTTGGCGAGGGTTCACCGCAACCGGTCTGA
D576A F	TCCGACCTGGTGTCTCGGAGAGGTCTGACGAC
D576A R	GTCGTCTGACCTCTCCGAGCACCAGGTCTGGA
P611A F	GCGACCCTGGGACCGGCCTTGGTG
P611A R	CACCAAGGCCGGTCCCAGGGTCTGC
E825A F	TCGCCGGTGGCGGGAGTCCGGTCTGC
E825A R	GCGACCGACTCCCGCCACCGGCGA
R852A F	CAGTTGGCCGGGGGAATCCGCCGC
R852A R	GCGGCGGATTCCCCCGCCAACCTG
plasR F	CCAAGCTTATGGCCTTGGTTGACGGTTTTC (<i>Hind</i> III)
plasR R	CGGGATCCTCAGAGAGTAATAAGACCC (<i>Bam</i> H I)
pTCR1	CCAAGCTTTCAGAACCAGGGAACGAAACC (<i>Hind</i> III)
pTCR2	AACAGCGGTGCTAGCAGGTGGTAG
pTCR3	CTACCACCTGCTAGCACCGCTGTT
pTCR4	CTGTGGTCTCGCGCCGCGGATCCAG
pTCR5	CTGGATCCGCGGCCGCGACCACAG
pTCR6	CGGAATTCCTTACGAGACCTCGAAAAAAGAGGG (<i>Eco</i> R I)
pKO-lasR1	CGAGCTCCCCGAACGGAAAAGT (<i>Sac</i> I)
pKO-lasR2	GAAGAACGTAGCGCTTCTTGCCTCTCAGGT
pKO-lasR3	ACCTGAGAGGCAAGAAGCGCTACGTTCTTC
pKO-lasR4	GCTCTAGATAACCATCGATTTCCATC (<i>Xba</i> I)
pCR1-F	CGGAATTCGGGAUGGACGCUAUGCUUGG (<i>Eco</i> R I)
pCR1-R	CAAGCTTAAAGGGACTTCGTTTCGACT (<i>Hind</i> III)
perRNA1-12 F	CGGAATTCGTTTGCAGGAAGCGGCGCTATCGCACCGAACTTAAA AGATCTTTGACAGCTAGC
perRNA1-12 R	CAAGCTTCACAAAAAAGCACCGACTCGGTG (<i>Hind</i> III)
EGFP F	CGGAATTCATGGTTTCGAAGGGCGAG (<i>Eco</i> R I)
EGFP R	CCCAAGCTTTTACTTGTACAGCTCGTC (<i>Hind</i> III)

lasR mRNA substrate

lasR 428-467	CGUUUCAUGGAGUCGGUCCAGCCGACCCUGUGGAUGCUC
lasR 330-369	GUGUAUGGGCUGACCAUGCCGUGCAUGGUGCGCGGGC
lasR 136-175	GGACUACGAGAACGCCUUCUUCGUCGGCAACUACCCGGCC
lasR 381-420	CUGAGCCUCAGCGUGGAAGCGGAGAACCAGGGCCGAGGCCA
lasR 435-474	CAUGGAGUCGGUCCAGCCGACCCUGUGGAUGCUCUAAAGGAC
lasR 648-687 (WT)	UCGGCGGAAGUUCGGUGCGAUAGCGCCGCUUCCUGCAAAC
100% mu	UCGGCGGAAGUUCGGUGCGAUAGCGCCGCUUCCUGCAAAC
PAM mu1	UCGGCUUCAGUUCGGUGCGAUAGCGCCGCUUCCUGCAAAC
PAM mu2	UCGGCGUAAGUUCGGUGUGACCUCGCGCCGCUUAGCGGCC
PAM mu3	UCGGCUGAAGUUCGGUGUGACCUCGCGCCGCUUAGCGGCC

PAM mu4	UCGGCUUAAGUUCGGUGUGACCUCGCGCGUAGCGGCC
PAM mu5	UCGGC ^{GG} UAGUUCGGUGUGACCUCGCGCGUAGCGGCC
PAM mu6	UCGGC ^{GG} GAGUUCGGUGUGACCUCGCGCGUAGCGGCC
PAM mu7	UCGGC ^{GG} CAGUUCGGUGUGACCUCGCGCGUAGCGGCC
Length mu1	UCGGCGGAAGUUCGGUGCGAACCUCCCGCGCGUAGCGGCC
Length mu2	UCGGCGGAAGUUCGGUGUGCCUCCCGCGCGUAGCGGCC
Length mu3	UCGGCGGAAGUUCGGUGUCCUCCCGCGCGUAGCGGCC
Length mu4	UCGGCGGAAGUUCGGUUUCCUCCCGCGCGUAGCGGCC
Length mu5	UCGGCGGAAGUUCGGUUUCCUCCCGCGCGUAGCGGCC
Length mu6	UCGGCGGAAGUUCGUGUUUCCUCCCGCGCGUAGCGGCC
Length mu7	UCGGCGGAAGUUCUUGUUUCCUCCCGCGCGUAGCGGCC
Length mu8	UCGGCGGAAGUUAUUGUUUCCUCCCGCGCGUAGCGGCC
Length mu9	UCGGCGGAAGUGAUUGUUUCCUCCCGCGCGUAGCGGCC
Length mu10	UCGGCGGAAGGGAUUGUUUCCUCCCGCGCGUAGCGGCC
Length mu11	UCGGCGGAAGUGAUUGUUUCCUCCCGCGCGUAGCGGCC
Length mu13	UCGGCGGACUGGAUUGUUUCCUCCCGCGCGUAGCGGCC
Mis mu1	UCGGCGGACGUUCGGUGUGAACCUCCCGCGCGUAGCGGCC
Mis mu2	UCGGCGGAUUUCGGUGUGAACCUCCCGCGCGUAGCGGCC
Mis mu3	UCGGCGGAAGUCGGUGUGAACCUCCCGCGCGUAGCGGCC
Mis mu4	UCGGCGGAAGUGCGGUGUGAACCUCCCGCGCGUAGCGGCC
Mis mu5	UCGGCGGAAGUUAGGUGUGAACCUCCCGCGCGUAGCGGCC
Mis mu6	UCGGCGGAAGUUCUGUGUGAACCUCCCGCGCGUAGCGGCC
Mis mu7	UCGGCGGAAGUUCGUUGUGAACCUCCCGCGCGUAGCGGCC
Mis mu8	UCGGCGGAAGUUCGGGGUGAACCUCCCGCGCGUAGCGGCC
Mis mu9	UCGGCGGAAGUUCGGUUUGAACCUCCCGCGCGUAGCGGCC
Mis mu10	UCGGCGGAAGUUCGGUGUUAACCUCCCGCGCGUAGCGGCC
Mis mu11	UCGGCGGAAGUUCGGUGUGCCUCCCGCGCGUAGCGGCC
crRNAs	
1-10	GUUCACUGCCGUAUAGGCAGCUAAGAAAAGAACAUGGCCCGG CUCGCGACGUUGCUGCGG
1-12	GUUCACUGCCGUAUAGGCAGCUAAGAAAGUUUGCAGGAAGCG GCGCUAUCGCACCGAACU
2-5	UUUCUUAGCUGCCUACACGGCAGUGAACAACCGCGCGCAACA GCUGUGCAUCGUCACCUU
2-6	UUUCUUAGCUGCCUACACGGCAGUGAACCGUCUCGAUGCUGC CGUCGAUGAGCGUCUUGU
2-18	UUUCUUAGCUGCCUACACGGCAGUGAACGACCAGAUCUGCC GCAGUACUGGCAGGACCG
2-19	UUUCUUAGCUGCCUACACGGCAGUGAACGUCGAGCCACUGU GUCGGCCAAAACCAGGUC

References

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