Supporting Information

Rediverting Electron Flux with an Engineered CRISPR-ddAsCpf1 System to Enhance Pollutant Degradation Capacity of *Shewanella oneidensis*

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This supporting information contains 12-page document, including 2 tables, 5 figures and this cover page.

MATERIALS AND METHODS

Strains, Plasmids and Cultivation Conditions. Both *E. coli* and *S. oneidensis* strains were cultured in liquid 2×YT medium or on its agar (1.5% w/v) aerobically at 37 ^{o}C and 30 ^{o}C, respectively. When necessary, tetracycline was used at 10 μg/mL in *E. coli* and 5 μg/mL in *S. oneidensis.* Chloramphenicol was used at 30 μg/mL in *E. coli* and 10 μg/ml in *S. oneidensis*. For the growth of *E. coli* WM3064 that has RP4 Tra function integrated into genome, 2,6-diaminopomelic acid (DAP) at 0.5 mM was supplemented to the culture medium[1]. Isopropyl β-d-1-thiogalactopyranoside (IPTG) was added to the medium at concentrations indicated.

DNA Analysis, Genetic Manipulation and Conjugation. Plasmid DNA was isolated from *E. coli* and *S. oneidensis* using OMEGA Plasmid Mini Kit (Omega Genetics Co., China). Enzymes for restriction digestion and Gibson mix were purchased from New England BioLabs (NEB, USA). Q5 High-Fidelity DNA polymerase was used for amplifying fragments for plasmid construction. MonAmp 2×Taq Mix (Monad Biotech Co., China) was used for PCR verification. Sanger DNA sequencing was performed on a 3730xl DNA Analyzer (General Biosystems Co., China).

E. coli strain was washed twice with ice-cold 10% glycerol and concentrated by 100-fold. Then, electroporation was performed with pre-chilled 2 mm gap electroporation cuvette (Bio-Rad) and electroporated at 2.5 kV with a Bio-Rad MicroPulser. One mL of 2-YT medium was added to the shocked cells and recovered for 1 h before plating on 2×YT agar with appropriate antibiotics. Hence, plasmids

were firstly transformed into the plasmid donor cell *E. coli* WM3064 (the DAP auxotroph) and then transferred into *S. oneidensis* via conjugation. Briefly, 1 ml of donor cells and 1 ml of recipient cells were mixed and collected by centrifugation at 4000 rpm for 10 min. Then, the cells were resuspended in 1 ml 2×YT medium containing DAP and plated on 2×YT agar (containing DAP) for conjugation (lasting 24-48 h) at 30 ^{o}C. The cells were then washed off from the agar plate with liquid 2×YT without DAP. An appropriate volume of the cells suspension was diluted and spread on 2×YT agar plates with appropriate antibiotics for selection of conjugative colonies. *S. oneidensis* MR-1 cells harboring a single-plasmid served as the recipient cell to generate the two-plasmid harboring *S. oneidensis* strains.

Table S1 Strains and Plasmids Used in This Work

strain or plasmid	relevant characteristics	ref.
strains		
Shewanella oneidensis MR-1	Wild type	lab stock
S. oneidensis GZ	*S. oneidensis* MR-1 with genome integrated GFP-lacZ cassette	lab stock
Escherichia coli WM3064	thrB1004 pro thi rpsL hsdS lacZΔM15 RP4-1360 Δ(araBAD)567 ΔdapA1341::[erm pir]	1
E. coli NEB10β	*Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- ϕ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL* (Str^R) *rph spoT1 Δ(mrr-hsdRMS-mcrBC)*	NEB
E. coli GZ	*E. coli* genome integrated with A GFP-*lacZ* cassette	lab stock
plasmids		
pEC01K-PLAC	p15A, Kan^R, oriV, trfA, lacI-P_{Lac}-GFPmut3b-T_1T_2	2
pMT-P_{Lac}-GFP	pMT backbone, Tet^R, IPTG inducible P_{Lac} promoter evaluation plasmid	this work
pXX55-ddAsCpf1	P15A origin, Kan^R, ddAsCpf1 encoding plasmid	3
pEAC01	pMT backbone, Tet^R, ddAsCpf1 driven by IPTG inducible P_{Lac} promoter	this work
pCN001	pBBR1 replicon, Cm^R	4
pECR-DR	pBBR1 replicon, Cm^R, an empty crRNA driven with only direct repeat sequence by P_{CN} promoter, used as control	this work
pECR-P1	crRNA plasmid targeting to GFP-P1	this work
pECR-NP1	crRNA plasmid targeting to GFP-NP1	this work
pECR-T1	crRNA plasmid targeting to GFP-T1	this work
pECR-T2	crRNA plasmid targeting to GFP-T2	this work
pECR-NT1	crRNA plasmid targeting to GFP-NT1	this work
pECR-sorB	crRNA plasmid targeting to gene *sorB*	this work
pECR-so0717	crRNA plasmid targeting to gene so*0717*	this work
pECR-napB	crRNA plasmid targeting to gene *napB*	this work
pECR-fccA	crRNA plasmid targeting to gene *fccA*	this work
pECR-dmsE	crRNA plasmid targeting to gene *dmsE*	this work
pECR-cctA	crRNA plasmid targeting to gene *cctA*	this work
pECR-so2930	crRNA plasmid targeting to gene so*2930*	this work
pECR-nrfA	crRNA plasmid targeting to gene *nrfA*	this work
pECR-dhc	crRNA plasmid targeting to gene *dhc*	this work
pECR-coxB	crRNA plasmid targeting to gene *coxB*	this work

Table S2. Primers Used in This Work

Primers	Sequences (5'-3')	Utilization
Blut-PMTPLAC F	CAGAATTTGCCTGGCGGCAGTAG	construct
Blut-PMTPLAC R	AGCTGTTTCCTGTGTGAAATTGTTATCCG	pEAC01
PMTPLAC-DCFP F	ATTTCACACAGGAAACAGCTATGACACAGTTCGAGGGCTTTACCAACCTG	
PMTPLAC-DCFP R	CTGCCGCCAGGCAAATTCTGGTACTAGTTCCTCAGCTCCTGGATG	
Con.PMTPLAC F	GCCAGTTGTTGTGCCACGC	
Con.PMTPLAC R	CATACACGGTGCCTGACTGCG	
SEQ.DCPF1 F	TACCGGCCGGTACAGAGACCTG	
SEQ.DCPF1 R	GCCATTAAACAGCTCGGCCTTGAAC	
crRNA-DR-T1T2 F	CAGGTGCTTTTTCACCCCAATTTCTACTCTTGTAGATGAGCCAGGCATCAAATAAAACG	Construct
BLU-PCN R	GGGGTGAAAAAGCACCTGTATACG	pECR-DR
crRNA-P1-T1T2 F	TCTACTCTTGTAGATCACTTTATGCTTCCGGCTCGTATAGCCAGGCATCAAATAAAACG	Construct
crRNA-NP1-T1T2 F	TCTACTCTTGTAGATCCACACAACATACGAGCCGGAAGAGCCAGGCATCAAATAAAACG	crRNA
crRNA-T1-T1T2 F	TCTACTCTTGTAGATACTGGAGTTGTCCCAATTCTTGTGAGCCAGGCATCAAATAAAACG	library
crRNA-T2-T1T2 F	TCTACTCTTGTAGATCAACGTCGTGACTGGGAAAACCCGAGCCAGGCATCAAATAAAACG	
crRNA-NT1-T1T2 F	TCTACTCTTGTAGATTGCCCATTAACATCACCATCTAAGAGCCAGGCATCAAATAAAACG	
crRNA-0716-T1T2 F	TCTACTCTTGTAGATACTGGGGCGTCGTTCGCCGCCGAGAGCCAGGCATCAAATAAAACG	
crRNA-0717-T1T2 F	TCTACTCTTGTAGATTCGTCAGTTTGCTTGTCCTGTGCGAGCCAGGCATCAAATAAAACG	
crRNA-0845-T1T2 F	TCTACTCTTGTAGATCCGACCAACCACCGCTTATCCCCGAGCCAGGCATCAAATAAAACG	
crRNA-0970-T1T2 F	TCTACTCTTGTAGATACGGTGAAATGGGTGGCTGCGATGAGCCAGGCATCAAATAAAACG	
crRNA-1427-T1T2 F	TCTACTCTTGTAGATATGTGCCATCGTAAAAATAACACGAGCCAGGCATCAAATAAAACG	
crRNA-2727-T1T2 F	TCTACTCTTGTAGATATCCGCGGCAAATGCGGTTGGCGGAGCCAGGCATCAAATAAAACG	
crRNA-2930-T1T2 F	TCTACTCTTGTAGATAGATGATGATCCCGCACCGGTTGGAGCCAGGCATCAAATAAAACG	
crRNA-3980-T1T2 F	TCTACTCTTGTAGATCATTAAGTGCATTGGTTGCCGCCGAGCCAGGCATCAAATAAAACG	
crRNA-4485-T1T2 F	TCTACTCTTGTAGATACCTTCTCAAGTACGGGTTATGCGAGCCAGGCATCAAATAAAACG	
crRNA-4606-T1T2 F	TCTACTCTTGTAGATTGTTACGCCAGGTGTCATGTTGTGAGCCAGGCATCAAATAAAACG	
Blt-DR R	ATCTACAAGAGTAGAAATTGGGGTG	
SEQ.PCN F	GCTCCTCTAGACTCGAGGAATTCTG	
CmR R	TTCAGGTTCATCATGCCGTTT	
primers for qRT-PCR		
q-ribBA F	GAAGGCGTAACCACAGG	
q-ribBA R	ATATGCCCAGGCTGAAC	
q-ribC F	TATTAGTCATACCGCACAGC	
q-ribC R	AACCGTTAGACTACAGCC	
q-ribD F	TTATGTCACCTTAGAACCCTG	
q-ribD R	TCTTCAACCGCCACCA	
q-ribE F	CGAAGCGAAGAATGCC	
q-ribE R	CACCAGGTACACGGACAA	
q-RibC1 F	AGCGTAAAGGCGATGA	
q-RibC1 R	TGAACCACGGTCAAACA	
q-mtrC F	TCGTTAGCCACGAAGTA	
q-mtrC R	ACCATCCGCAAACTCT	
q-omcA F	GGATACGGCGTTGAAGAT	
q-omcA R	TGGTATCCGTTCCATTCC	
q-cymA F	TCAACACTGCCATACTCG	
q-cymA R	GACACCTTTGTGGCAAT	
q-aceF F	ATGAAGGAAGACGTGCAG	
q-aceF R	TTAGGTGCCGCAATCAC	
q-pflB F	TACCATCACTTCCCACGAC	
q-pflB R	CATACGAATACCACCGTT	
q-gltA F	TTTGCATTATCGCGTACAGT	
q-gltA R	CTCGCCAGTATAAAGCTGAC	

q-pta F	TAGGTGCCCTGCTGCTTACT
q-pta R	GAGGTTTGCCAAGTGTTGGT
q-ackA F	CTTAGAAGACTCACGCATC
q-ackA R	CTTGAATTTGCGCTGCT
q-RecA F	TGAAAACCCAGCTATAGCC
q-RecA R	CCTCGACATTGTCATCATC
q-0716 F	TATCCCGTGGACCCTCAAT
q-0716 R	ATCACCGCGATTTTGGGT
q-0717 F	GCTCAATGTGAGTCATGCCAT
q-0717 R	ACCTGTGATTGCATTAACGGATC
q-0845 F	GCCGCGATTGTATTAGCCATC
q-0845 R	ACGAATATCCGTCACCGC
q-0970 F	TCGATGACTCTATCCGTAAGAGC
q-0970 R	CAGGTACATCGATTTGTTTCGC
q-1427 F	CCATGCAGCTAAAGATCCGG
q-1427 R	GGTTATGACAATCGATGCAGGTC
q-2727 F	CTGAAATGGACGCAGTACACA
q-2727 R	GGCAGCTTTCACAAGTAGGTT
q-2930 F	CCTTTAATATTGACTCGACCTTGC
q-2930 R	GAGAAATTAAGCACAGTGTCCTTC
q-3980 F	GTCATATGCCTAAAGTGACTAGCC
q-3980 R	CGCTCATTAGTGACGCCAAC
q-4485 F	CGAGCTGAATCAAGCCATCC
q-4485 R	GCATTATCGCCAAAGTGGTTG
q-4606 F	ATGCCATGATCAACCACAG
q-4606 R	GCGTTTTAGTTGCTGGAATAGC

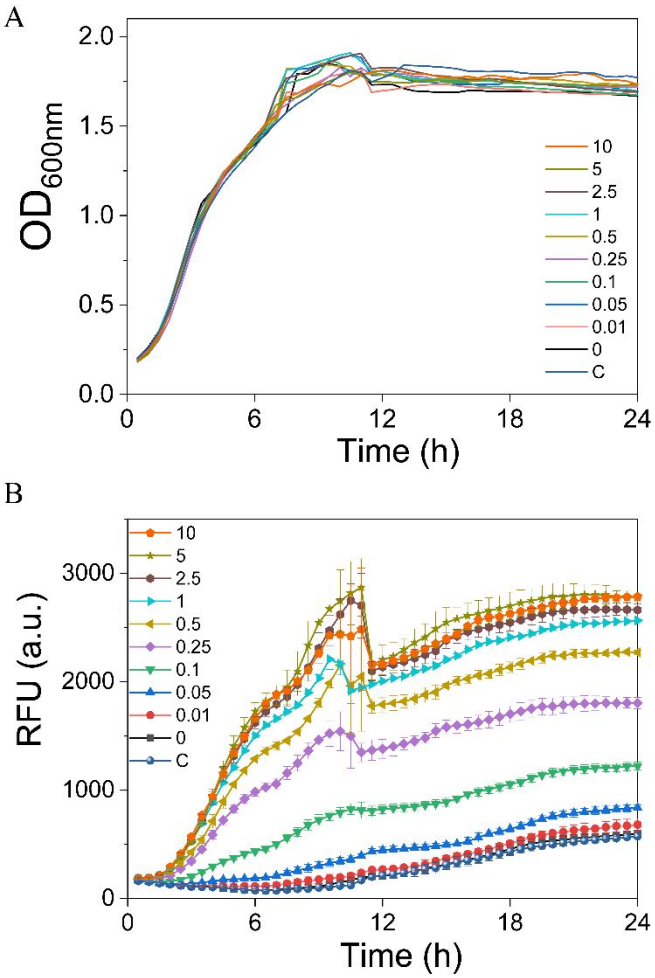

Figure S1. Growth curves (A), and GFP fluorescence (B) of *S. oneidensis* MR-1 carrying the plasmid encoding an IPTG inducible promoter controlled GFP at various IPTG inducer concentrations (0-10 mM). C: *S. oneidensis* MR-1 harboring an empty plasmid as the control. Three samples were evaluated and the standard errors are indicated.

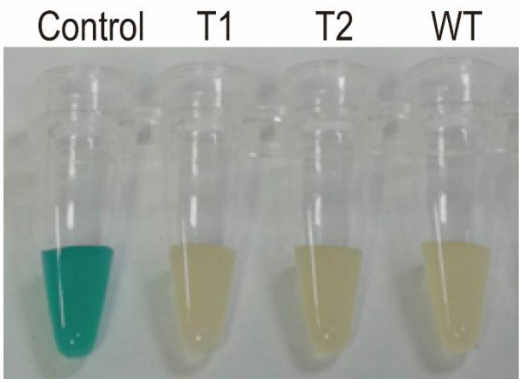

Figure S2. Evaluation of the crRNA-T2 targeting *lacZ* ORF downstream of GFP gene in the GFP-LacZ tandem cassette. Control: plasmid pECR-DR with an empty crRNA; WT: *S. oneidensis* MR-1 harboring both pMT and pCN001empty plasmids.

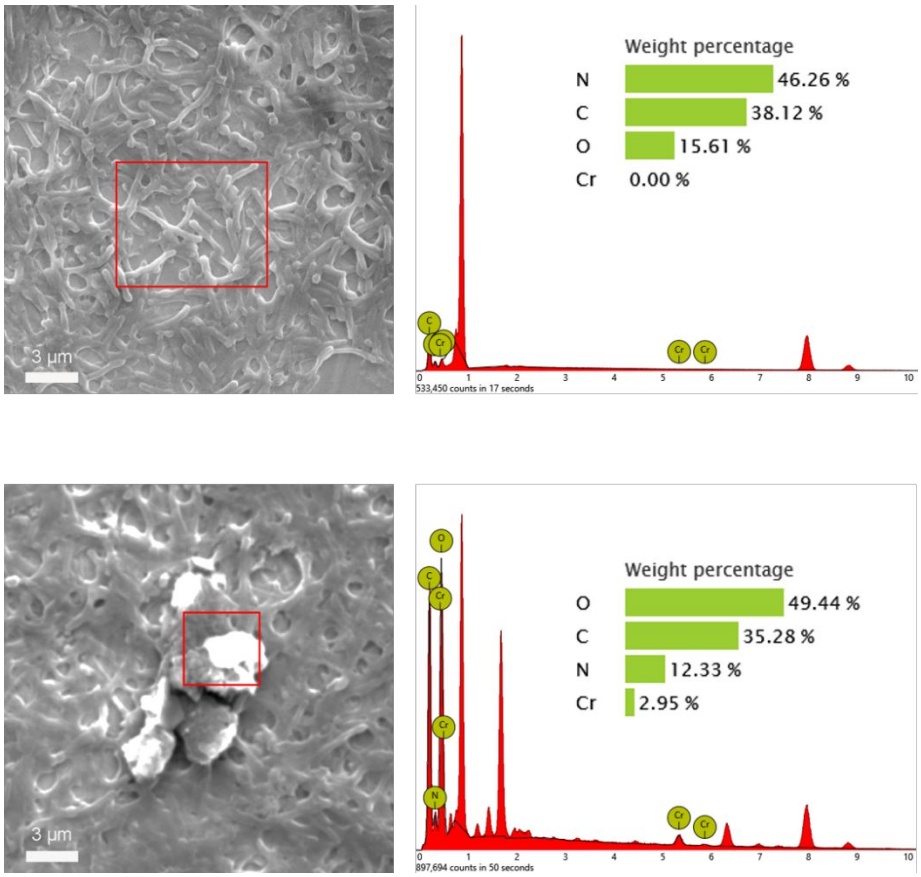

Figure S3. SEM and EDS results of the participate on the cell surface of *S. oneidensis* carrying pECR-dmsE before (Upper panel) and after (Lower panel) exposure to Cr(VI).

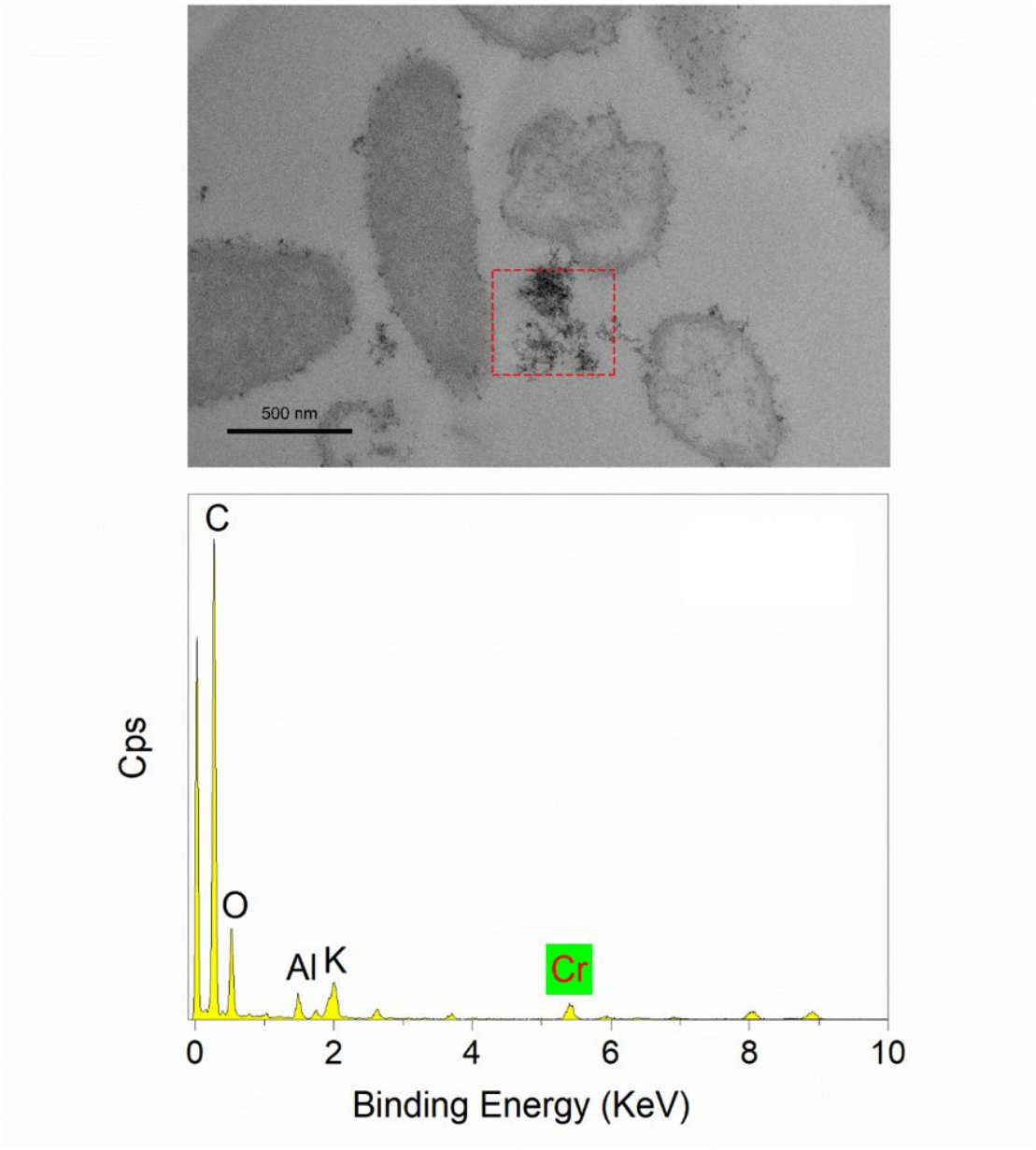

Figure S4. TEM and EDS results of the formed participates on cell surface of *S. oneidensis* carrying pECR-dmsE after loading with Cr(VI).

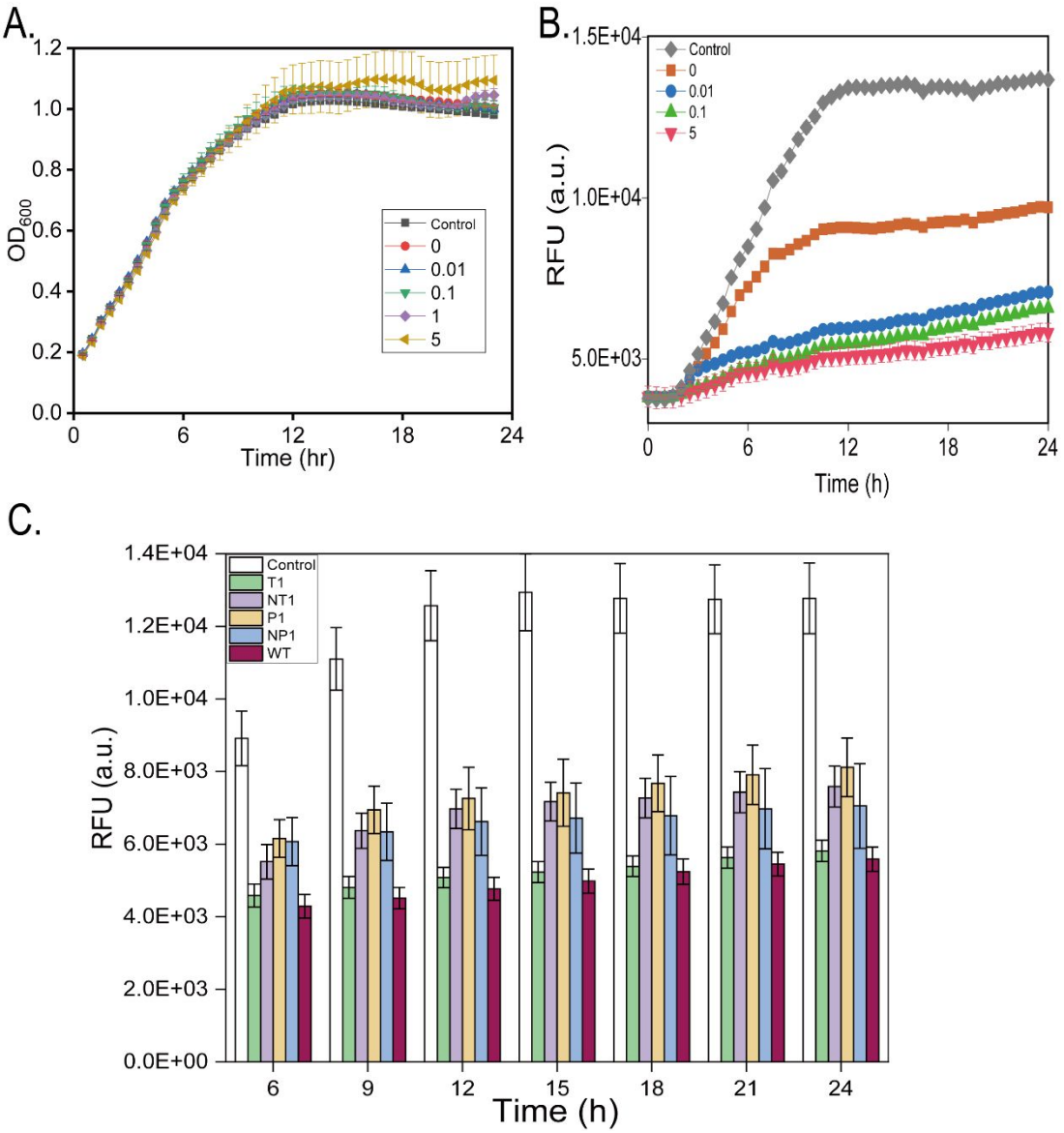

Figure S5. Evaluation of the engineered CRISPR-ddAsCpf1 system for gene interference in *E. coli.* (A) Evaluation of various IPTG inducer concentrations (mM) on cell growth of *E. coli*. Evaluation of gene interference ability to various IPTG concentrations (B) and variously targeted crRNAs (C) Control: *E. coli* carrying the empty crRNA plasmid pECR-DR; WT: *E. coli* NEB10β. For all experiments, three samples were evaluated and the standard errors are indicated.

References

1. Dehio, C.; Meyer, M. Maintenance of broad-host-range incompatibility group P and group Q plasmids and transposition of Tn5 in *Bartonella henselae* following conjugal plasmid transfer from *Escherichia coli*. *J. Bacteriol.* **1997,** *179* (2), 538-540.
2. Tang, Q.; Lu, T.; Liu, S. J. Developing a synthetic biology toolkit for *Comamonas testosteroni*, an emerging cellular chassis for bioremediation. *ACS. Synth. Biol.* **2018**, 7(7), 1753-1762.
3. Zhang, X.; Wang, J.; Cheng, Q.; Zheng, X.; Zhao, G.; Wang, J. Multiplex gene regulation by CRISPR-ddCpf1. *Cell. Discov.* **2017,** *3*, 17018.
4. Tang, Q.; Lu, T.; Liu, S. J. Engineering the bacterium *Comamonas testosteroni* CNB-1: Plasmid curing and genetic manipulation. *Biochem. Eng. J.* **2018,** *133*, 74-82.