

**Supplementary information for:**

**A novel dual-*cre* motif enables two-way autoregulation of CcpA in  
*Clostridium acetobutylicum***

Running title: CcpA autoregulation

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**Table S1** Primers used in this study.

Primer name	Sequence (5'-3')	Description
<i>P<sub>ccpA</sub>Amut</i> -for	taagtataggggatataacaatagaaaatttgcggt	forward primer used for overexpression
<i>P<sub>ccpA</sub>Amut</i> -rev	aacgcaaatttctattgttatatcccctatactta	reverse primer used for overexpression
<i>P<sub>ccpA</sub>-PstI</i> -for	taatcttttcataaaaaatcactcctataa	forward primer used for overexpression
<i>P<sub>ccpA</sub>-lacZ</i> -rev	ttataggagtgattttatgagaaagatta	reverse primer used for overexpression
<i>lacZ-P<sub>ccpA</sub></i> -for	ataggaataatctttctcactcatgtaaataac	forward primer used for overexpression
<i>lacZ-SmaI</i> -rev	atggagttattacatgagatgagaaagattatt	reverse primer used for overexpression
<i>lacZ-ccpA</i> -for	ataggaataatctttctcactcatgtaaataac	forward primer used for overexpression
<i>ccpA-lacZ</i> -rev	atggagttattacatgagatgagaaagattatt	reverse primer used for overexpression
<i>ccpA<sub>mut</sub></i> -for	cagatttagatatacgaaggagaaggactatattag agttcttaaggaga	forward primer used for overexpression
<i>ccpA<sub>mut</sub></i> -rev	tctccttaagaactctaataatagtccttctccttcgatat atctaaatctg	reverse primer used for overexpression
<i>P<sub>ihl</sub>-PstI</i> -for	aaaactgcagttttaacaaaatata	forward primer used for overexpression
<i>P<sub>ihl</sub>-ccpA</i> -rev	ttaatagaggcagccattctaactaacctcctaa	reverse primer used for overexpression
<i>ccpA-P<sub>ihl</sub></i> -for	ttaggaggttagtgaatggctgcctctattaa	forward primer used for overexpression
<i>ccpA-EcoRI</i> -rev	ccggaattcttattacatgaatc	reverse primer used for overexpression
M-L1-for	tataggtatataacaatag	forward primer used for EMSA
M-L1-rev	ctattgttatataacctata	reverse primer used for EMSA
M-L2-for	tatatattatataacaatag	forward primer used for EMSA
M-L2-rev	ctattgttatataatata	reverse primer used for EMSA
M-L3-for	tatatggtatataacaatag	forward primer used for EMSA
M-L3-rev	ctattgttatataacctata	reverse primer used for EMSA
M-L4-for	tatatgtgatataacaatag	forward primer used for EMSA
M-L4-rev	ctattgttatataccatata	reverse primer used for EMSA
M-L5-for	tatatggtgataacaatag	forward primer used for EMSA
M-L5-rev	ctattgttataacaatata	reverse primer used for EMSA
M-L6-for	tatatgtagataacaatag	forward primer used for EMSA
M-L6-rev	ctattgttatcaaatata	reverse primer used for EMSA
M-R1-for	tatatggtat gtaacaatag	forward primer used for EMSA
M-R1-rev	ctattgttac ataacaatata	reverse primer used for EMSA
M-R2-for	tatatgttata gaacaatag	forward primer used for EMSA
M-R2-rev	ctattgttctataacaatata	reverse primer used for EMSA
M-R3-for	tatatgttatat gacaatag	forward primer used for EMSA
M-R3-rev	ctattgtcatataacaatata	reverse primer used for EMSA
M-R4-for	tatatgttatata gcaatag	forward primer used for EMSA
M-R4-rev	ctattgtctataacaatata	reverse primer used for EMSA
M-R5-for	tatatgttatataaa aaatag	forward primer used for EMSA
M-R5-rev	ctattttatataacaatata	reverse primer used for EMSA
M-R6-for	tatatgttatataac gatag	forward primer used for EMSA
M-R6-rev	ctatcgttatataacaatata	reverse primer used for EMSA
M-L1-R6-for	tataggttatataacgatag	forward primer used for EMSA

M-L1-R6-rev	ctatcgttatataacctata	reverse primer used for EMSA
qlacZ-for	atatgctatcatggagtaatggatagttagatt	forward qRT-PCR primer for lacZ
qlacZ-rev	atatggatacctttgtacgcacatctctattggat	reverse qRT-PCR primer for lacZ
qCAC2679-for	ttaaacgaacctgcaacttcta	forward qRT-PCR primer for CAC2679
qCAC2679-rev	cccttagcccatttattctaca	reverse qRT-PCR primer for CAC2679
qCAC3037-for-1	gtaatggaagcaattaa	forward qRT-PCR primer for CAC3037
qCAC3037-rev-1	tttcttcgatatactaaa	reverse qRT-PCR primer for CAC3037
qCAC3037-for-2	ggtggttgaaggctataga	forward qRT-PCR primer for CAC3037
qCAC3037-rev-2	gttgctctattgtagtaagc	reverse qRT-PCR primer for CAC3037
ccpA-outer-Dw	tgtaaataactccatcaaccatct	For 5'-RACE
ccpA-inner-Dw	tctccttaagaactctaataat	For 5'-RACE

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Figure S1

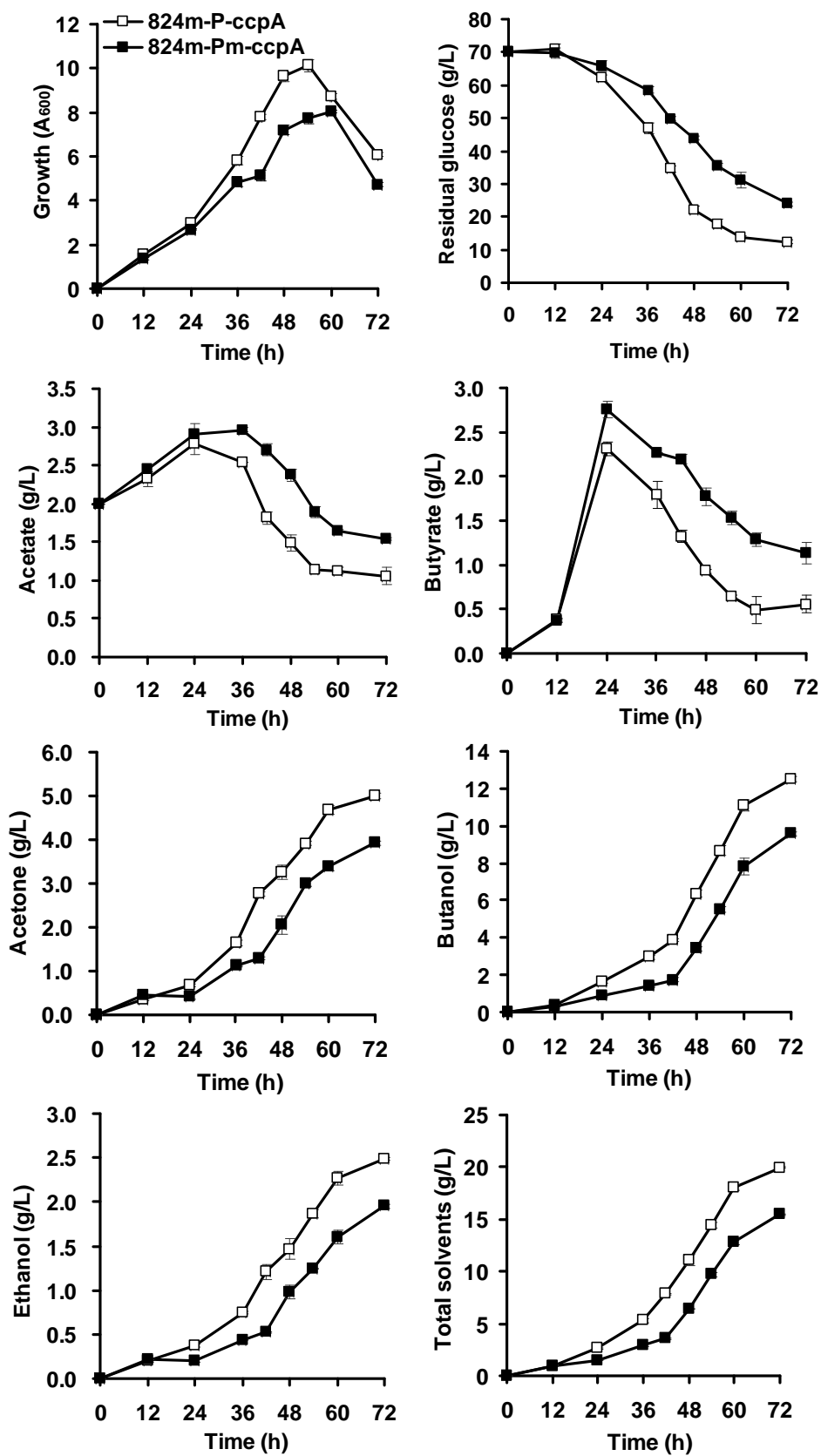


Figure S1. The phenotypic changes caused by mutation of the *cre-p* sited in the

***ccpA* promoter region.** Fermentations were carried out using glucose as the sole carbon resource. 824m-P-*ccpA*: the *ccpA*-mutated *C. acetobutylicum* strain harboring an expression plasmid that carried the *ccpA* gene and its native promoter. 824m-Pm-*ccpA*: same as the 824m-P-*ccpA* strain except that the *cre-p* site in the promoter was mutated. Total solvents represent the sum of acetone, butanol and ethanol. The mean of three independent biological replicates and the standard deviation are shown.

Figure S2

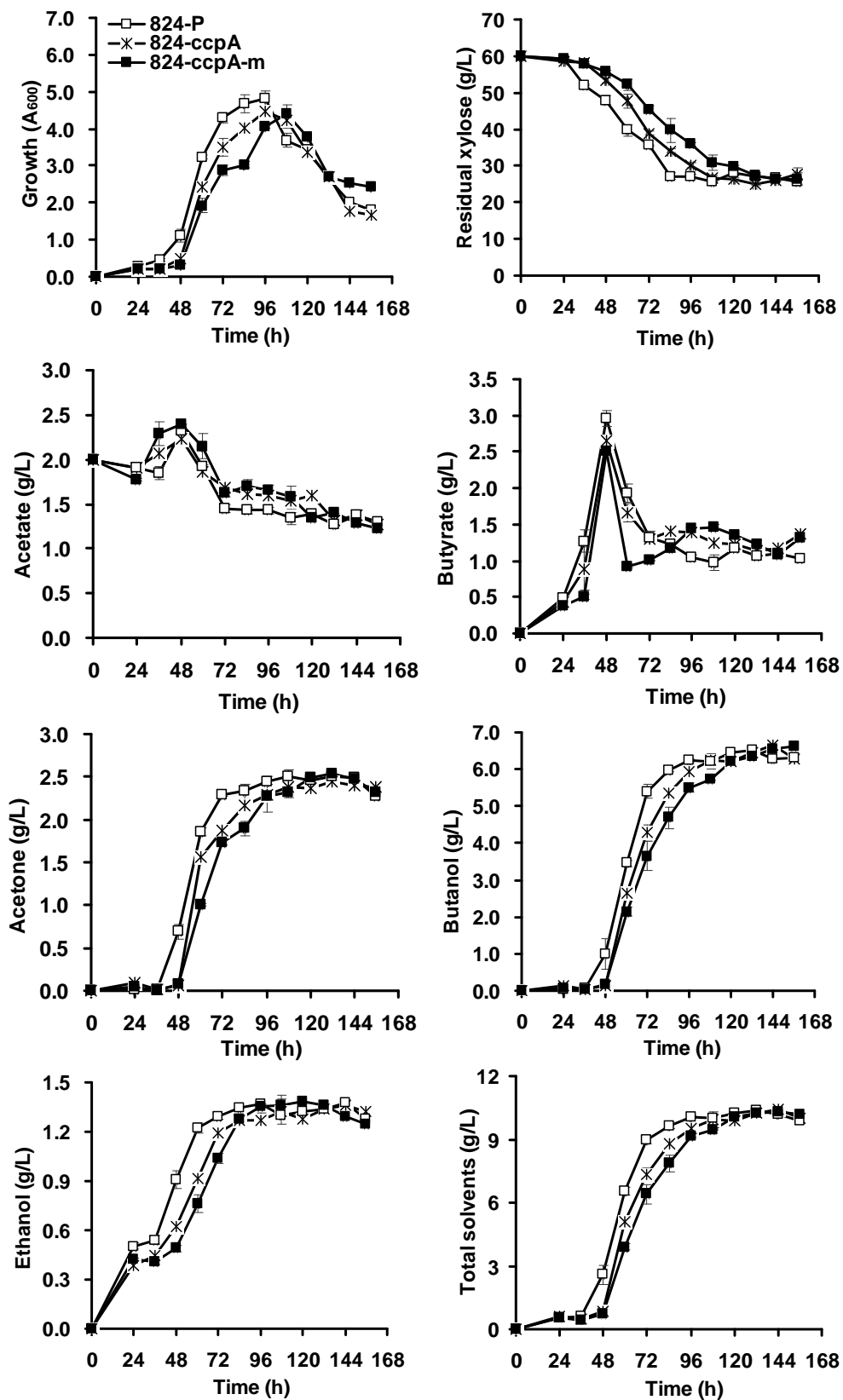
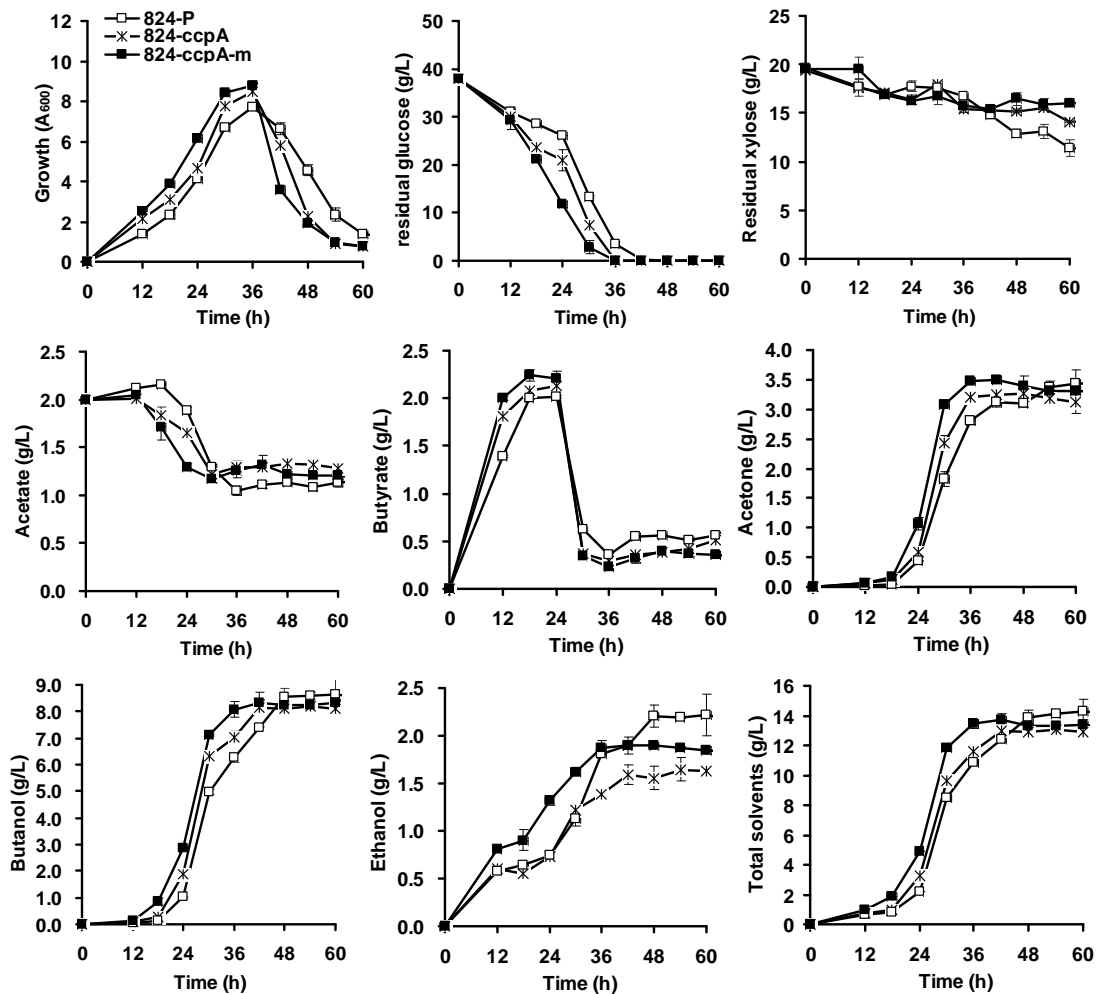


Figure S2. The fermentation profiles of the *ccpA*-overexpressed strains and

**wild-type strain using xylose as the sole carbon resource.** 824-P: the wild-type *C. acetobutylicum* strain harboring a control plasmid pIMP1-P<sub>thl</sub>. 824-ccpA: the *C. acetobutylicum* strain harboring the plasmid pIMP1-P<sub>thl</sub>-ccpA that carried wild-type *ccpA* gene for overexpression. 824-ccpA-m: the *C. acetobutylicum* strain harboring the plasmid pIMP1-P<sub>thl</sub>-ccpA<sub>mut</sub> that carried the *cre*-ORF-mutated *ccpA* gene for overexpression. Total solvents represent the sum of acetone, butanol and ethanol. The mean of three independent biological replicates and the standard deviation are shown.

**Figure S3**



**Figure S3. The fermentation profiles of the *ccpA*-overexpressed strains and wild-type strain using the mixture of glucose and xylose as carbon resources.** 824-P: the wild-type *C. acetobutylicum* strain harboring a control plasmid pIMP1- $P_{thl}$ . 824-ccpA: the *C. acetobutylicum* strain harboring the plasmid pIMP1- $P_{thl}$ -ccpA that carried wild-type *ccpA* gene for overexpression. 824-ccpA-m: the *C. acetobutylicum* strain harboring the plasmid pIMP1- $P_{thl}$ -ccpA<sub>mut</sub> that carried the *cre*-ORF-mutated *ccpA* gene for overexpression. Total solvents represent the sum of acetone, butanol and ethanol. The mean of three independent biological replicates and the standard deviation are shown.