

TABLE S1 Primers used in this study

Gene disruption in <i>C. acetobutylicum</i>		
<i>rex</i> -inactivated mutant (CAC2713)	IBS: 5'- AAAACTCGAGATAATTATCCTTAGGAGTCCTTGCTGTGCGCCCAGATAGGGTG -3' EBS1d: 5'- CAGATTGTACAAATGTGGTGATAACAGATAAGTCCTTGCTTATAACTTACCTTTCTTTGT -3' EBS2: 5'- TGAACGCAAGTTTCTAATTTTCGATTACTCCTCGATAGAGGAAAGTGTCT -3' EBS: 5'- CGAAATTAGAACTTGCGTTCAGTAAAC -3'	The primer IBS, EBS1d and EBS2,EBS, which were designed using the InGex Intron Prediction Program (www.Sigma-Aldrich.com/Targetronaccess), were used to amplify the targetron target sequence.
Confirmation of gene disruption in <i>C. acetobutylicum</i>		
<i>rex</i> -inactivated mutant (CAC2713)	5'- GTGGATAAGAAAAAAAACATATC -3' 5'- CTAATCTCGGCTTTTATTAC -3'	
Gene complementation in <i>C. acetobutylicum</i>		
<i>rex</i> -complemented strain (CAC2712)	5'- <u>aactgcag</u> GTGGATAAGAAAAAAAACATATC -3' 5'- <u>acgcgtc</u> gacCTAATCTCGGCTTTTATTAC -3'	Introduced restriction sites (PstI for the 5'-end and SalI for the 3'-end) are underlined.
Quantitative RT-PCR analysis of gene expression		

<i>ldh</i> (CAC0267)	5'- CGGTGGACTTGCATCTGAG -3' 5'- GCTGCTCCCCGCTGTTATT -3'	
<i>nadA</i> (CAC1025)	5'- AAAAGCTAAGCACCCCTAATG-3' 5'- TACAGAAGCCATCCCATA-3'	
<i>nadB</i> (CAC1024)	5'- ATTATTGGCACAGGGGTTG -3' 5'- GTGTCATCAACAAATAGAGGC -3'	
<i>nadC</i> (CAC1023)	5'- GCTTAGAGGGCACAGGGG -3' 5'- CGTCCAAGGTTTCCGTTTC -3'	
<i>asrT</i> (CAC1512)	5'- GCATTTATTACCTGTGGATTG -3' 5'- TATACTCCTTTCTTCTGCCTTG -3'	
<i>asrA</i> (CAC1511)	5'- AAAGAAGCAGAAGGACCTAAG -3' 5'- CACTCGCATCATACATATCAGC -3'	
<i>asrB</i> (CAC1510)	5'- AGGTGTCGTGGAGTATTTG -3' 5'- CAGATGCTTCCTTTACATTCTG -3'	
<i>asrC</i> (CAC1509)	5'- ATTATGGAGACGGTTCTGTTTC -3' 5'- GGCATACACGATTTCCCAC -3'	

<i>crt</i> (CAC2712)	5'- TTTAGGAGGCGGATGCG -3' 5'- TGCCATGCCCATTCCAAC -3'	
<i>bcd</i> (CAC2711)	5'- GCAATGAAAACCTTTGATGG -3' 5'- GTATGGAAGTCCTGCTTG -3'	
<i>etfB</i> (CAC2710)	5'- CTTTGGCTATGGGTGCTG -3' 5'- CTATTTCTGGTCCAACCTGAG -3'	
<i>etfA</i> (CAC2709)	5'- TATTAGTTGCTGGTGGTAGAGG -3' 5'- ATCTGAATCTTGCATACCTGC -3'	
<i>hbd</i> (CAC2708)	5'- CGGAACAGTTGACCTTAATATG -3' 5'- TCTTTTAGTTGCTGATGCCAC -3'	
<i>thlA</i> (CAC2873)	5'- GCAGTAAGAACAGCGATTGG -3' 5'- TGCCTGTCTTGCTGGATTC -3'	
<i>ptb</i> (CAC3076)	5'- TATCAGAAGAAGCAGCAC-3' 5'- CAACTAAGATTCCCTCC-3'	
<i>buk</i> (CAC3075)	5'- AAAGATAAACGGCAAAGGC -3' 5'- TTACATACATGCTCGTTGTACG -3'	
<i>adhE2</i> (CAP0035)	5'- GTTTCGGTTATGGCTACG -3' 5'- TTCAATGTCGTTAGTCCC -3'	

<i>adhE1</i> (CAP0162)	5'- GATGCAGCCGTTAAGAGTG -3' 5'- AGTGAGGGACCACCAGTTG -3'	
<i>ctfA</i> (CAP0163)	5'- TCTGGCTTAGGTGGTGTAC -3' 5'- CATCGGCTGTAAGAGGTAG -3'	
<i>ctfB</i> (CAP0164)	5'- TCAACTTGTAAACTTAGGTGTAGG -3' 5'- CACGGATTAGTGAAAACGAAAC -3'	
<i>adc</i> (CAP0165)	5'- ACTTCGCCTGCATTTTC -3' 5'- GCTCTGGCACAACCTTTAC -3'	
<i>bdhA</i> (CAC3299)	5'- ACTTTCAGGAGTAGAGCCAAATC -3' 5'- TCCCATGTATCGCCATC -3'	
<i>bdhB</i> (CAC3298)	5'- CAGGATAGAATGGCAGAAGC -3' 5'- AAGACTTGAAGCCCACATTAG -3'	
<i>fprA1</i> (CAC1027)	5'- CTGCCCCTAACCTTCATTG -3' 5'- TCTTCTCCACCCTCATCC -3'	
<i>nrer</i> (CAC2448)	5'- TCCTCACGCTGACGAAAT -3' 5'- GCTCTATACCTAAGATACCTCCAC -3'	
<i>fpA2</i> (CAC2449)	5'- TTATTGCCTCAAATGCTGG -3' 5'- ATGGCGAACTTTAGGACC -3'	

<i>dfx</i> (CAC2450)	5'- TAGATGTTTCTGTAGGTTCCG -3' 5'- CTGCTTTCCATAGGCTGTG -3'	
<i>gd</i> (CAC2777)	5'- CAAAGTCCTACTTGGCTTCTC -3' 5'- GCCTACTACTATGTTTCCGTC -3'	
<i>rd</i> (CAC2778)	5'- GATCCAGCCGAAGGTGATC -3' 5'- CTTCAGATGGCTCAAATTGATC -3'	
<i>rbr3B</i> (CAC3597)	5'- AATGCCCGTATGTGGTG-3' 5'- GCAACTTCAGGGTATCCTTCTC -3'	
<i>rbr3A</i> (CAC3598)	5'- AATGCCCTGTATGTGGTGC-3' 5'- TGCTTGTCTTGCCATTGC -3'	
CAC2679	5'- TGTTGTATGGAGAAGGCTGGGA-3' 5'- CACTTCCCTTTAAGGCATCGCGTA-3'	Internal control
Protein overexpression and purification		
pET (CAC2713)	5'- <u>cgcgatcc</u> GTGGATAAGAAAAAAAAACATATC -3' 5'- <u>acgctcgac</u> CTAATCTCGGCTTTTATTCAC -3'	Introduced restriction sites (BamHI for the 5'-end and SalI for the 3'-end) are underlined.
pET(CAC2713) (Q47K)	5'-GCAGATACGCAAAGACCTAAATTG -3' 5'-CAATTTAGGTCTTTGCGTATCTGC -3'	
Amplification of 180-bp DNA fragments from the upstream region of genes for EMSA		

CAC0267 (<i>ldh</i>)	5'- AACTTCAATAGATGGGCTC -3' 5'- TTATCCCCTCCAATTACTA -3'	
CAC1025 (<i>nadA</i>)	5'- AGACAATCACATACACCCC -3' 5'- GTAAACAAGATACAGTAAAT -3'	
CAC1512 (<i>asrT</i>)	5'- ATCTAGACAGCTGAAGC -3' 5'- CATCTTCGAACACTTTA -3'	
CAC2712 (<i>crt</i>)	5'- GCTGTTTATTGCAAGAAGTGAATAAAAAGC -3' 5'- TTTTATTGTTAATATTATAACAAAGACG -3'	
CAC2873 (<i>thlA</i>)	5'- ATGCATAAGTTTAATTTTTTTTG -3' 5'- TCTAACTAACCTCCTAAATT -3'	
CAC3076 (<i>ptb</i>)	5'-GGAGGCATAATATCAGCGGCTG-3' 5'-CATTAAAACCTCTTAATCACTG-3'	
CAP0035 (<i>adhE2</i>)	5'- TAGTATTTGGCAATAAAAATAG -3' 5'-TTACATTATTTACTTCTAATG -3'	
Cbei_1009 (<i>pflB</i>)	5'- CGCGAACTTATAGAAA -3' 5'- TTAGTTTTTCCTCCTTT -3'	
Cbei_2181 (<i>adhA</i>)	5'- TTTCTGAGGAATTACG -3' 5'- CCTAAAATTTGCTCAT -3'	

Cbei_1722 (<i>adhA2</i>)	5'- AAGTTAGAACCTTGTA -3' 5'- TACCTCCTAAAATTCA -3'	
Cbei_1464 (<i>butA</i>)	5'- CCTAAATAACGCAAAA -3' 5'- CACCATTAAGTGAAGTTTA -3'	
Cbei_0327 (<i>hydB</i>)	5'- AAAAGAAGTGGAATG -3' 5'- CTCCCATGAATAATTT -3'	
Cbei_4319 (<i>fld</i>)	5'-GCACAAATTATACAATC-3' 5'-GTTCGCAAGTGCTTCTAT-3'	
Cbei_1014 (<i>ldh</i>)	5'- ATCTTCTGCGTAAAAC -3' 5'- TTGCTCATAACAACCTT -3'	Negative control
NT0278 (<i>ctfA</i>)	5'- CTAAATATTTAAAAAAT -3' 5'- TAACTACACCCCATG -3'	
CTC01488 (<i>frdA</i>)	5'- GTAGTTCTTAATTAGAG -3' 5'- CATTTTCCCCCTATCTT -3'	
CBO0246 (<i>maeB</i>)	5'- GTTAAGCATAAAAATAT -3' 5'- CACCATTAAGTGAAGTTTA -3'	
CPF_0728 (<i>noxE</i>)	5'- AAATTCTTTGTTTAAAG-3'	

	5'- TATATTTCCACCTTTC -3'	
CLJU_c39440 (<i>bcd2</i>)	5'-GATAACGATAACATTG-3' 5'-GTATTTCTTCAAAAAGGTC-3'	
CLJU_c37670 (<i>codH</i>)	5'- CACACGCAATTGCATA -3' 5'- GTAATAAATACAATTAT -3'	
Ccarb_5267 (<i>narB</i>)	5'- CCATGATATACTTACAAT -3' 5'- GGCGCAATAATTACATG -3'	
Ccarb_4501 (<i>narK</i>)	5'- GTATAGTGATATACTT -3' 5'- GCAAACCCTACAAAAAAC -3'	
Ccarb_2944 (<i>codH</i>)	5'- CAAATTGGATTTTGAA -3' 5'- GTATATAAAAATTACA -3'	
Ccarb_5251 (<i>grdIH</i>)	5'- CAAATTTCTATAGCATGAAATC -3' 5'- GTTGAAATTGTCTAAATAGTAC -3'	
Ccarb_1246 (<i>crt</i>)	5'- AAACAATCTGCATTTGACCA -3' 5'- ACCCCATCTTATCCCTCCT -3'	Negative control
CAC2712 (<i>crt</i>)T ₅ -G ₅	5'- TTTGCTGATTGCAAGAAGTGAATAAAAAGC -3' 5'- TTTTTATTGTTAATATTATCACAAAGACG -3'	

CAC2712 (<i>crt</i>)A ₁₄ -C ₁₄	5'- TTTGCTGATTGCAAGAAGTGAATAAAAAGC -3' 5'- TTTTATTGTGAATATTATAACAAAGACG -3'	
CAC2712 (<i>crt</i>) T ₅ -G ₅ A ₁₄ -C ₁₄	5'-TTTGCTGATTGCAAGAAGTGAATAAAAAGC -3' 5'- TTTTATTGTGAATATTATCACAAAGACG -3'	

TABLE S2 Reconstructed Rex regulons in clostridia.

First Gene ID	Operon	Candidate Rex-binding sites	Position ^a	Score ^b	TSS position ^c
<i>C. acetobutylicum</i> ATCC 824					
CAC2712	<i>crt-bcd-etfB-etfA-hbd</i>	TTGTTATAATATTAACAA	-45	5.6	-126
CAC3076	<i>ptb-buk</i>	ACGTTAATCATTTAACAT	-52	4.8	-55
CAC0267	<i>ldh</i>	TAGTTAATAAATTAACAA	-98	5.6	-97
CAP0035	<i>adhE2</i>	TTGTTAAATGTTTGACAA	-262	4.9	-159,-215
CAC2873	<i>thlA</i>	TTGTGTTTTTTTTTAACAA	-161	4.6	-105
CAC1025	<i>nadABC</i>	TTGATAAATATCTAACAA	-110	5.3	-40
CAC1512	<i>asrTABC</i>	TTGTTATATTTTTTAACAA	-42	5.5	-86
<i>C. beijerinckii</i> NCIMB 8052					
Cbei_4319	<i>fld-pfor</i>	AAGTTAATTATTTAACAA	-37	5.5	-61
Cbei_1464	<i>butA</i>	ATGTGAATTAATTAACAA	-41	5.3	-120
Cbei_0321	<i>crt-bcd-etfBA</i>	TTGTTATATTATTAACAA	-87	5.5	-168
Cbei_0325	<i>hbd</i>	AAGTTAATAATGTAACAT	-146	4.8	-48
Cbei_0325	<i>hbd</i>	AAGATAATTAATTAACAA	-126	5.3	-48
Cbei_0411	<i>thlA</i>	TTGTTTAAATTTTAACAA	-118	5.4	-57
Cbei_0411	<i>thlA</i>	ATGTTAATAATTTAACAA	-58	5.7	-57
Cbei_0327	<i>hydB</i>	TTGTTATAATTTTAACAA	-84	5.6	-33
Cbei_0327	<i>hydB</i>	ATGTTAATAATTTATCAT	-287	5.3	-33
Cbei_1722	<i>adhA2</i>	TTGATAAAAATAATAACAT	-99	5.3	-272
Cbei_2181	<i>adhA</i>	TTGATAAAAATAATCACAA	-129	5.1	-243
Cbei_0203	<i>ptb-buk</i>	TTGATATAAAATTTAACAA	-74	5.6	-159
Cbei_3630	<i>thlA2</i>	TTGTTAAAAAAAATAACAA	-48	5.8	-42
Cbei_1009	<i>pflBA</i>	TTGATTAAAAATTAACAA	-52	5.4	-85,-546
<i>C. botulinum</i> A str, ATCC 3502					

CBO3202	<i>crt-hbd-thl-bcd-ETFBA</i>	TAGATAAAATTTTATCAT	-136	5	-316
CBO3202	<i>crt-hbd-thl-bcd-ETFBA</i>	TTGTTAAAATAATAGCTA	-93	4.8	-316
CBO1643	<i>adhA</i>	TAGTTATTATTTTATCAT	-261	4.8	-86,-460
CBO1519	<i>ldh</i>	ATGTTAATTAATTAACATA	-161	5.3	-259
CBO2217	<i>butA</i>	ATGTTAATTAATTAACAT	-51	5.5	-55,-363
CBO1579	<i>alsT-noxE-yeeE</i>	TTGAGAGAAAATTAACAA	-144	5	-98
CBO0345	<i>adhE2</i>	ATGATAAATATTTAACAA	-178	5.7	-293
CBO0345	<i>adhE2</i>	TTGTTAAAATAAATAACAA	-121	5.8	-293
CBO0246	<i>maeB</i>	TTGTTAATAATATAACAA	-145	5.7	-50,-350
<i>C. butyricum</i> 5521					
CBY_3041	<i>crt-bcd-ETFBA-hbd</i>	TTGTTATAATATTAACAA	-98	5.6	-286
CBY_2919	<i>ptb-buk</i>	TTGATATAATTTTAACAA	-55	5.4	-196
CBY_1290	<i>thlA</i>	ATGTTAATAATTTAACGA	-81	5.4	-30
CBY_1290	<i>thlA</i>	TTGTCTAAAATTTAACAA	-140	5.3	-30
CBY_3751	<i>adhA</i>	ATGATAAAAATATTACAA	-122	5	-32
CBY_3747	<i>adhA2</i>	TTGACAAAAAAATAACAA	-92	5.4	-122
CBY_3643	<i>fld-pfor</i>	AAGTTAATTATTTAACAA	-37	5.5	-63
CBY_3753	<i>adhE2</i>	GTGTTAAAAAAATAACAA	-104	5.5	-20,-320
CBY_3753	<i>adhE2</i>	TTGACAAAAAAATAACAA	-146	5	-20,-320
CBY_2357	<i>maeB</i>	TTGTTAAAAAAGTAACAA	-145	5.4	-227
CBY_2472	<i>butA</i>	ATGTGAATTAATTAACAA	-41	5.3	-144
CBY_3325	<i>narAB</i>	TTATTAATTTTTTAACAA	-170	4.89	-166
CBY_3325	<i>narAB</i>	TTGTTAAAAAAAGAACAA	-149	5.02	-166
<i>C. kluyveri</i> DSM 555					
CKL_0454	<i>crt-bcd-ETFBA-hbd</i>	TTGTGATATTATTAACAA	-64	5.1	-97
<i>C. novyi</i> NT					

NT01CX_0604	<i>hbd-thlA</i>	ATGATATATATTTAACAA	-233	5.4	-278
NT01CX_0604	<i>hbd-thlA</i>	AAGTTAAAAAATTAACAA	-46	5.6	-278
NT01CX_0474	<i>crt-bcd-etfBA-hbd</i>	TTGTTACTATATTAACAA	-69	5.2	-117
NT01CX_2324	<i>bcd-etfBA</i>	GTGTTTTTTTTTTAACAA	-114	4.7	-52
NT01CX_0464	<i>hydB</i>	ATGTTACATATTTAACAA	-97	5.3	-106
NT01CX_0468	<i>adhA</i>	TTGTTAATAAAAATAACAA	-61	5.7	-53
NT01CX_0309	<i>alsT-noxE-yeeE</i>	TTGAGAGAAATTTAACAA	-30	5.1	-196
NT01CX_0278	<i>ctfAB</i>	ATGACAAAAATTTAACAA	-90	5.4	-53,-400
<i>C. perfringens</i> ATCC 13124					
CPF_2585	<i>crt-bcd-etfBA-hbd</i>	TTGTTACAAAATTAACAA	-124	5.5	-161
CPF_2657	<i>ptb-buk</i>	GTGTTTAAAAATTTAACAA	-190	5.2	-117
CPF_2657	<i>ptb-buk</i>	TTGTTATAAATTTAACAA	-43	5.7	-117
CPF_2460	<i>thlA</i>	TTGATAAATTTTTACCTA	-35	4.7	-55
CPF_2460	<i>thlA</i>	TTGTTTAAAAATTTAACAA	-115	5.6	-55
CPF_0098	<i>ldh</i>	TTGTTATATAATTAACCTT	-64	5.1	-42,-342
CPF_2855	<i>adhE2</i>	TTGATAAAAAAATCACAA	-286	5.2	-225
CPF_2855	<i>adhE2</i>	TAGTTAAAAAATAACGA	-266	5.3	-225
CPF_0728	<i>noxE</i>	TAGATAAAAAATTTAACAA	-60	5.6	-221
CPF_2052	<i>narAB-noxA</i>	ATGATAAATTTTTAACAA	-47	5.5	-214
<i>C. tetani</i> E88					
CTC01847	<i>frdA2</i>	TAGTTAATATTTTAACAA	-40	5.5	-33
CTC01178	<i>noxE-yeeE</i>	TTGAAAGAAATTTAACAA	-141	5.1	-59,-438
CTC02427	<i>crt-bcd-etfBA-hbd</i>	TTGTTATAAATTCACAA	-245	5	-88
CTC02427	<i>crt-bcd-etfBA-hbd</i>	TTGTTATAATATTAACAA	-104	5.6	-88
CTC00312	<i>thlA</i>	TTGTTAATTTTTTAACCTA	-64	5.3	-77
CTC00710	<i>bcd-etfBA</i>	AAGTTAGTTATTTAACAA	-64	5.1	-20

CTC02422	<i>adhA</i>	AAGTTAAGTTATTAACAA	-157	4.8	-67
CTC02422	<i>adhA</i>	ATGTTAAAAATATATCAA	-50	5.4	-67
CTC02417	<i>hydB</i>	CTGTTAATAAATTAACAA	-84	5.3	-38
CTC02546	<i>ptb-buk</i>	TAGTTAAAAAGATATCAA	-48	4.8	-73
CTC01366	<i>adhE2</i>	TTGTTATATAATTAACAT	-139	5.5	-225
CTC01488	<i>frdA</i>	TTGACAAAATATTAACAA	-147	5.4	-172
<i>C. cellulovorans</i> 743B					
Clocel_1811	<i>pflBA</i>	ATGTTAATTATTTAACAT	-68	5.27	-228
Clocel_2402	<i>adhE2</i>	TTGTTAAATTAATAACAA	-175	5.48	-40,-515
Clocel_2976	<i>crt-bcd-etfBA-hbd</i>	TTGTTAATTTAATAACAA	-105	5.37	-70
Clocel_2976	<i>crt-bcd-etfBA-hbd</i>	TTGTTAATTTAATAACAA	-40	5.37	-70
<i>C. carboxidivorans</i> P7					
Ccarb_1085	<i>adhA</i>	TTGTGAAAAATATAACCA	-184	4.74	-96
Ccarb_2944	<i>codH-cooC-fhs-fchA-folD-metF-lpdA-cooC-acsDCEB</i>	ATGTTTAATTTTTAACGT	-193	4.57	-95,-498,-841 , -1289
Ccarb_4269	<i>ptb-buk</i>	ATGATTAAAAAATTTACAA	-1	4.81	-57,-462
Ccarb_4321	<i>adhE2</i>	TTGTTAAAAAAATAACGA	-290	5.23	-160,-488
Ccarb_4321	<i>adhE2</i>	TTGACAAATAATAACTA	-195	4.57	-160,-488
Ccarb_4500	<i>asrTABC</i>	TTGTGATATATTTATCAT	-192	4.82	-169
Ccarb_5154	<i>crt</i>	AAGTTAAATTATTAACGA	-140	4.9	-23,-235
Ccarb_5154	<i>crt</i>	ATGTTAATAATTTAACTA	-117	5.28	-23,-235
Ccarb_5251	<i>grdIH</i>	TTGATAATTTAATAACAA	-145	5.26	-149
Ccarb_4501	<i>narK</i>	AAGTTAAATAATTAACAA	-52	5.44	-34
Ccarb_4501	<i>narK</i>	TTGAAATAAATATAATAA	-30	4.5	-34
Ccarb_5267	<i>narAB-noxA</i>	AAGTTAAATAATTAACAA	-93	5.44	-54
<i>C. ljungdahlii</i> DSM 13528					
CLJU_c18470	<i>adhA</i>	TAGATAATATTTTAACAA	-59	5.31	-58

CLJU_c23220	<i>butA</i>	TAGTGAATATATTAACAG	-69	4.51	-26
CLJU_c23770	<i>asrABC-narKAB-noxA</i>	ATGTTAAATATATAACAA	-69	5.46	-70
CLJU_c23770	<i>asrABC-narKAB-noxA</i>	TTGATAATTTTTTAAAAA	29	4.77	-70
CLJU_c27840	<i>grdIH</i>	CTGTTATAAAAATAACAA	-175	5.13	-160
CLJU_c27840	<i>grdIH</i>	GTGTTAAAATTTAAACAA	-92	4.7	-160
CLJU_c37670	<i>codH-cooC-fhs-fchA-folD-metF-lpdA-cooC-acsDCEB</i>	ATGTTTATTTTTTAACAT	-185	4.89	-112
CLJU_c39440	<i>bcd2-fldA-bcd-etfBA</i>	TTGTTATAAAAATACACAA	-29	4.75	-262

^a Positions of the 5' end of Rex sites are given relative to the translational start.

^b Scores of candidate sites are the sum of positional nucleotide weights.

^c The transcriptional start sites (TSS) of all the candidate Rex target operons are obtained based on prediction of the potential -10 and -35 promoter elements using the SoftBerry-BPROM (<http://linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb>), except for CAC2712 (*crt*; Boynton et al., 1996), CAC3076 (*ptb*; Walter et al., 1993), CAP0035 (*adhE2*; Ref. 41), and CAC2873 (*thlA*; Stim-Herndon et al., 1995) genes whose TSS have been determined previously. Positions of the TSS are given relative to the translational start.

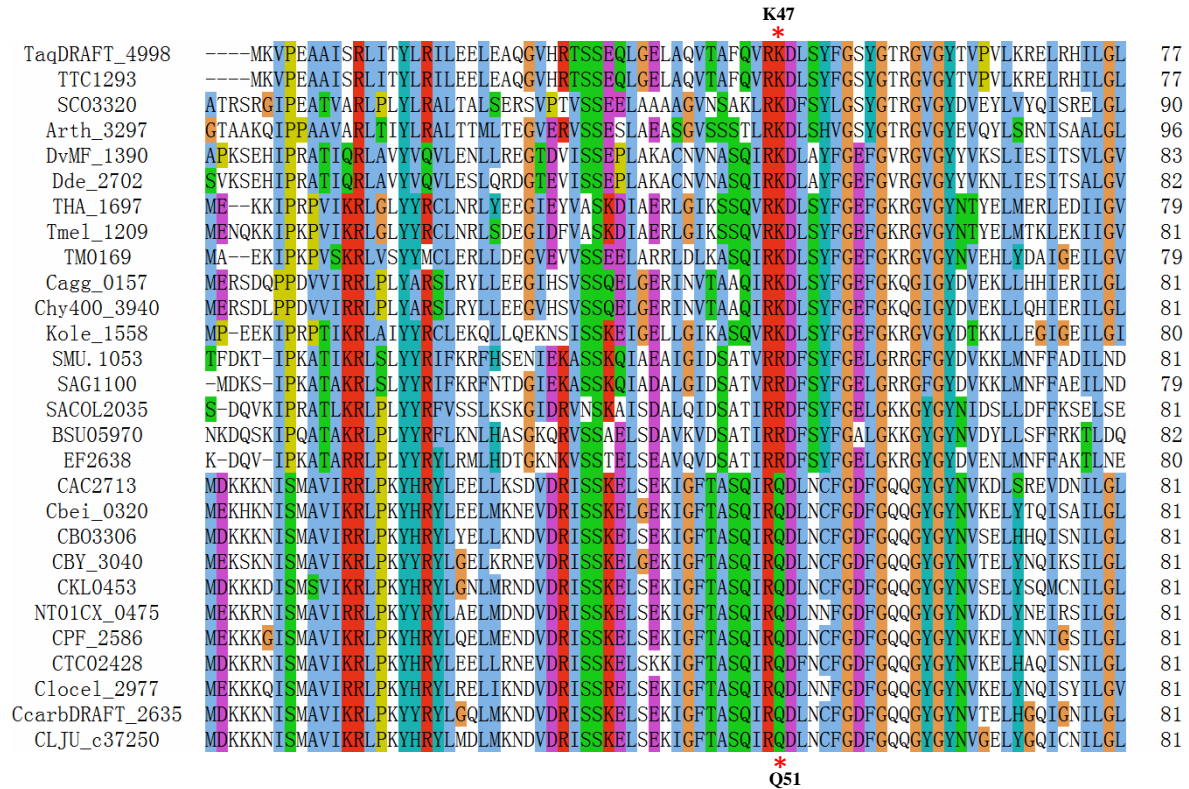


FIG S1 Sequence alignment of N-terminal DNA-binding domain of Rex proteins from clostridia and other Gram-positive bacteria including *Thermus aquaticus* Y51MC23 (TaqDRAFT_4998), *Thermus thermophilus* HB27 (TTC1293), *Streptomyces coelicolor* A3(2) (SCO3320), *Arthrobacter* sp. FB24 (Arth_3297), *Desulfovibrio vulgaris* str. 'Miyazaki F' (DvMF_1390), *Desulfovibrio desulfuricans* G20 (Dde_2702), *Thermosiphon africanus* TCF52B (THA_1697), *Thermosiphon melanesiensis* BI429 (Tme1_1209), *Thermotoga maritima* MSB8 (TM0169), *Chloroflexus aggregans* DSM 9485 (Cagg_0157), *Chloroflexus aurantiacus* Y-400-fl (Chy400_3940), *Kosmotoga olearia* TBF 19.5.1 (Kole_1558), *Streptococcus mutans* UA159 (SMU.1053), *Streptococcus agalactiae* 2603 V/R (SAG1100), *Staphylococcus aureus* subsp. *aureus* COL (SACOL2035), *Bacillus subtilis* 168 (BSU05970), *Enterococcus faecalis* strain V583 (EF2638), *Clostridium acetobutylicum* ATCC 824 (CAC2713), *Clostridium beijerinckii* NCIMB 8052 (Cbei_0320), *Clostridium botulinum* A str, ATCC 3502 (CBO3306), *Clostridium butyricum* 5521 (CBY_3040), *Clostridium kluyveri* DSM 555 (CKL0453), *Clostridium novyi* NT (NT01CX_0475), *Clostridium perfringens* ATCC 13124 (CPF_2586), *Clostridium tetani* E88 (CTC02428), *Clostridium cellulovorans* 743B (Cloce1_2977), *Clostridium carboxidivorans* P7 (Ccarb_2635), and *Clostridium ljungdahlii* DSM 13528 (CLJU_c37250).

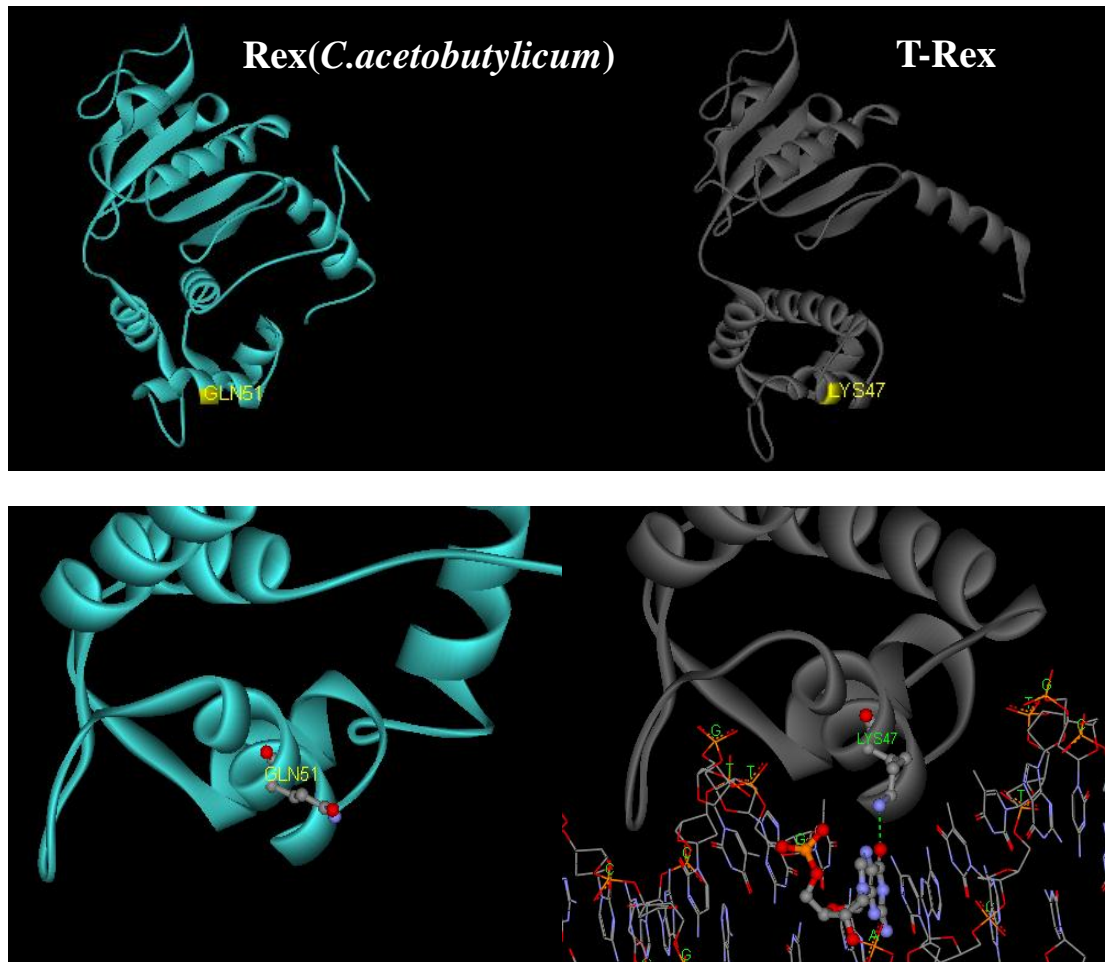


FIG S2 Three-dimensional homology model of *C. acetobutylicum* Rex protein. The model was generated based on the known tertiary structure of the *T. aquaticus* Rex regulator (T-Rex) in complex with its DNA operator (Protein Data Bank [PDB] accession number 3IKT) using the software Discovery Studio 2.1. The Gln51 residue of *C. acetobutylicum* Rex and Lys47 of T-Rex are highlighted in yellow and shown with ball-and-stick representation. The hydrogen bond between Lys47 and guanine is shown by green dashes.

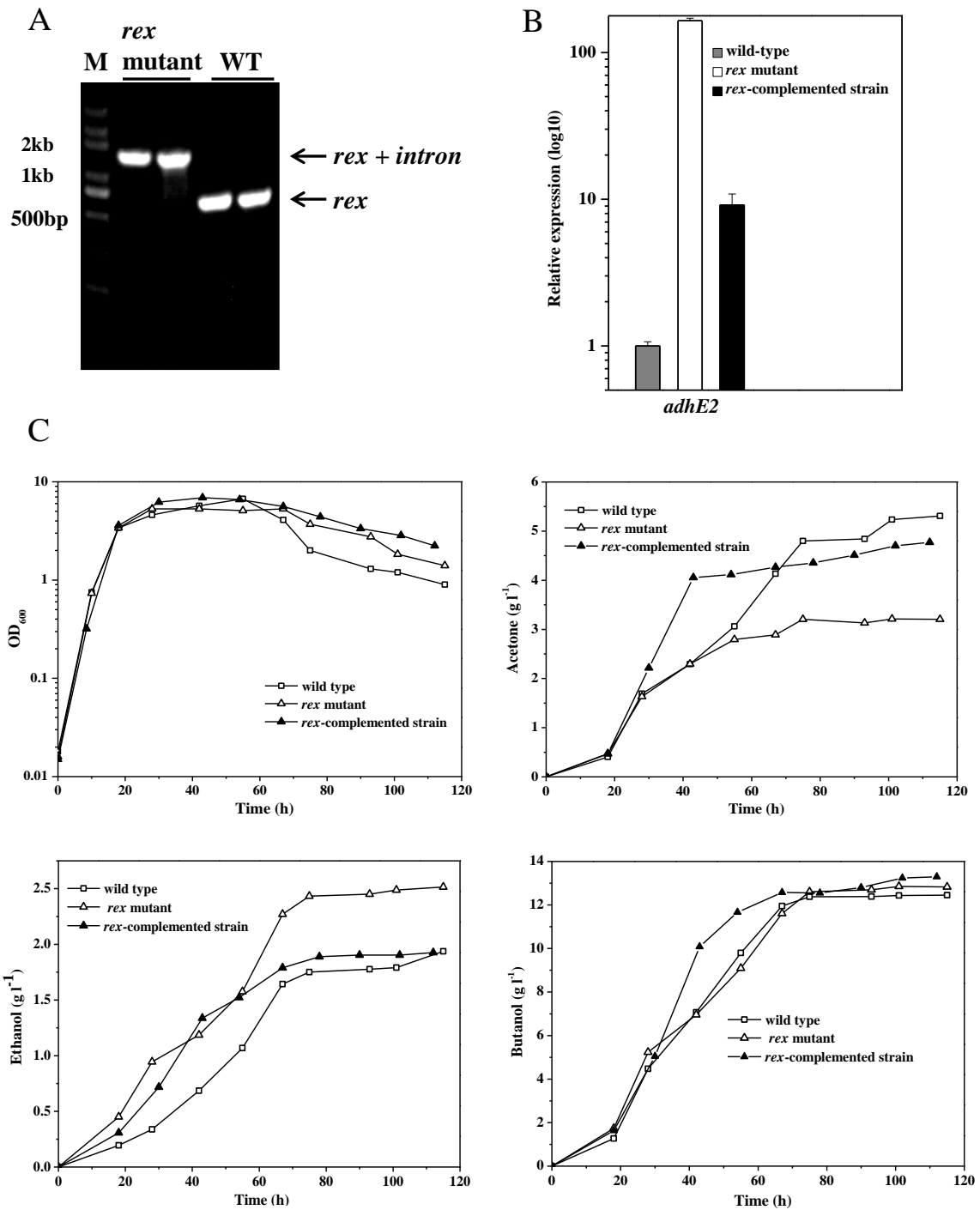


FIG S3 Validation of the genotype of *C. acetobutylicum* *rex*-inactivated mutant. The mutant with intron insertion in *rex* gene was confirmed by PCR (A). Complementation of the *rex*-inactivated mutant by using a plasmid construct constitutively expressing *rex* resulted in a reduced expression level of *adhE2* gene compared to the gene expression in the *rex*-inactivated mutant (B) and a fermentation profile similar to that of the wild-type strain (C).

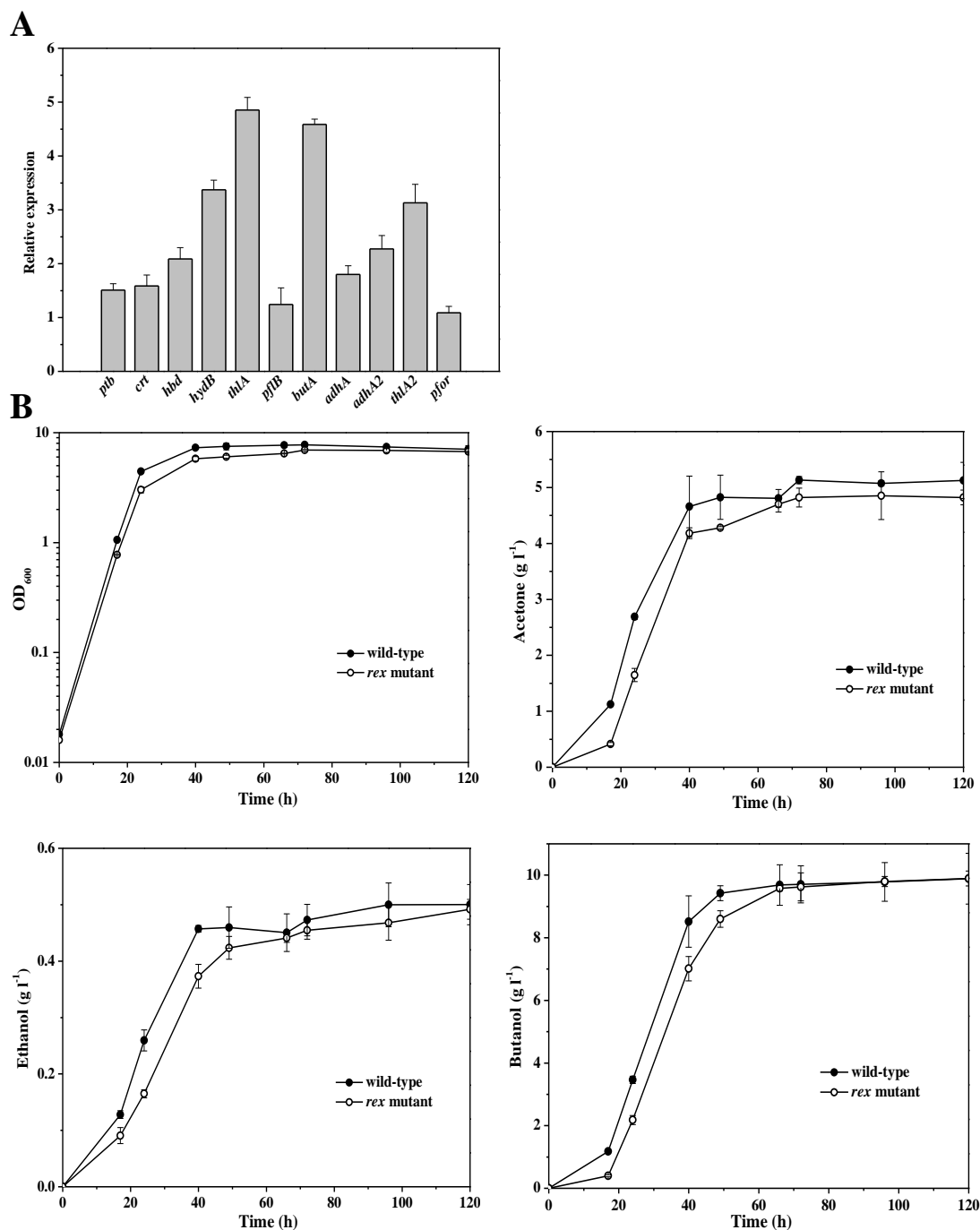


FIG S4 Effect of *rex* inactivation on transcript levels of the predicted Rex targets (A) and fermentation product formation of *C. beijerinckii* (B). The *C. beijerinckii* wild-type and *rex*-inactivated mutant strains were grown in the XHP2 minimal medium (Xiao et al., 2012). The data points represent the average of three independent experiments.