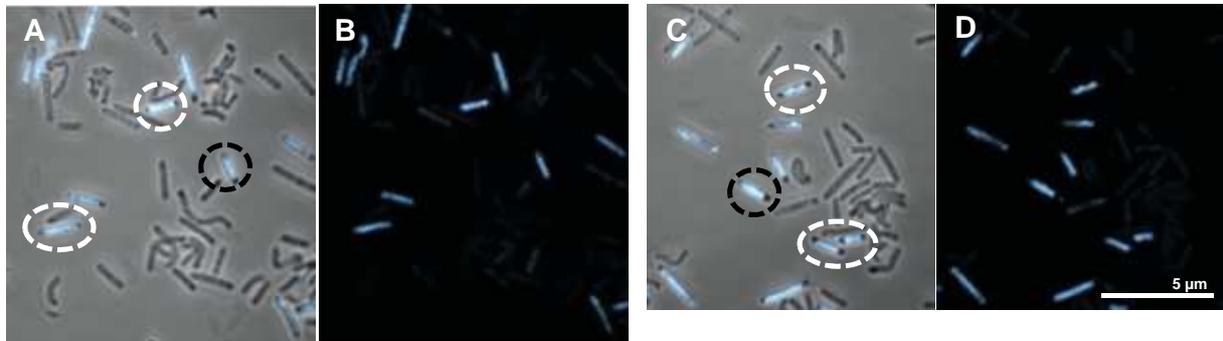


## SUPPLEMENTAL MATERIALS

### Supplemental Figure



**FIG. S1.** FK01 *C. acetobutylicum* cells stained with DAPI. Image overlays of the phase-contrast images with the DAPI images (A & C). DAPI images (B & D). Dashed white circles indicate cells with two phase-dark areas on either pole of the cell. Dashed black circles indicate cells with a single phase-dark area at one pole of the cell. DAPI stains all cells, but stained some cells more than others.

**Table S1.** PCR primers.

<b>Name</b>	<b>Sequence (5' → 3')</b>	<b>Description</b>
RecU-Seq-F	GCCGAAACATTCGGTTTCATCCCA	Amplify region for sequencing
RecU-Seq-R	TCAACCTTTCGCACCAGATGATGG	Amplify region for sequencing
SigF-F	TTTGAGAGATGACGGAATAAT	Amplify <i>sigF</i> gene fragment
SigF-R	CAGCATATTTGCGACCTGAG	Amplify <i>sigF</i> gene fragment
SigF-KO-F	GGTTGTATGGGACTCGTAAAGGCT	Flanking confirmation primer
SigF-KO-R	TCCCTTATAGCCTTCAGTATTCTT	Flanking confirmation primer
SigF-probe-F	TTTGAGAGATGACGGAATAA	Amplify Southern probe
SigF-probe-R	ACTGCATACTAGTAGTAGAT	Amplify Southern probe
SpoIIA-F	AAATTTCCGGGAAATTCTAGATAA	Amplify <i>spoIIA</i> operon
SpoIIA-R	GTAGTTCCCTCCCTAGATATTTTT	Amplify <i>spoIIA</i> operon
SpoIIA-KI-F	CCTTTGCCAAGAAGCTTGGAGAGAAC	Flanking confirmation primer
SpoIIA-KI-R	TCTGAAACGAGCTGACCCACATCA	Flanking confirmation primer
TET-R	AGATACCTGCGTTGCTTTGGTCTG	Primer within Tet <sup>r</sup> cassette
TET-Seq-F	TACTACCGGTGAACCTGTTTGCCA	Amplify region for sequencing
TET-Seq-R	AGGAGCATCGTTGTATGCTCGTGA	Amplify region for sequencing
TH-F	GGAATGGCGTGTGTGTTAGCCAAA	Amplify Cm/Th <sup>r</sup> cassette
TH-R	TCACACAGGAAACAGCTATGACCA	Amplify Cm/Th <sup>r</sup> cassette
TH-frag-F	AACCGCCATTCAGAGTTTAGGACG	Amplify Cm/Th <sup>r</sup> fragment
TH-frag-R	CGGAGCATTGGCTTTCCCTCCAT	Amplify Cm/Th <sup>r</sup> fragment
CAC3571-F	CCCAGGTAAACCAATAATGCCAGG	Q-PCR housekeeping primer
CAC3571-R	GCTCCAAGTATAGGCTTCTTTCCC	Q-PCR housekeeping primer
<i>csfB</i> -F	CTTGCATTATATGCAGAAAGCCTC	Q-PCR primer for <i>csfB</i>
<i>csfB</i> -R	CCACATTGTAGCTTAAC TAATCTCT	Q-PCR primer for <i>csfB</i>
<i>gpr</i> -F	TATGCATAGATGCACTAGCCGCGA	Q-PCR primer for <i>gpr</i>
<i>gpr</i> -R	CCAGGAGATATTCCAGTGTGCCA	Q-PCR primer for <i>gpr</i>
<i>spoIIP</i> -F	AATGTATGTGCGGTGGGCGATGAA	Q-PCR primer for <i>spoIIP</i>
<i>spoIIP</i> -R	GTGTTTACCTGAACGCGTATAAC	Q-PCR primer for <i>spoIIP</i>
<i>sigG</i> -F	GGAATGGAAACACAACCTGCCAGAG	Q-PCR primer for <i>sigG</i>
<i>sigG</i> -R	AGCCCTATACACCCAACCTGAA	Q-PCR primer for <i>sigG</i>
<i>spoIIAA</i> -F	TGGACAGCTCAGGAATTGGAGT	Q-PCR primer for <i>spoIIAA</i>
<i>spoIIAA</i> -R	GGTCCTACTACACATATACTACCGCC	Q-PCR primer for <i>spoIIAA</i>
<i>spoIIAB</i> -F	TTGCGTCAGAGCTTGATCCCACTT	Q-PCR primer for <i>spoIIAB</i>
<i>spoIIAB</i> -R	CCCTATTCCCTTCGTCCATAACCTC	Q-PCR primer for <i>spoIIAB</i>
<i>lonB</i> -F	GCCGGTGTAAGTATTGCATGTGCT	Q-PCR primer for <i>lonB</i>
<i>lonB</i> -R	TGCTGCCGCTGTAACCTTAGAACT	Q-PCR primer for <i>lonB</i>
<i>spoIIR</i> -F	GCTAGCAAAGGTTGATTTCCCGGT	Q-PCR primer for <i>spoIIR</i>
<i>spoIIR</i> -R	AACACAGCGCGGAAACATAACAC	Q-PCR primer for <i>spoIIR</i>
<i>spoIIE</i> -F	GCAGTGAGTTTCAATTTGCTATAAGTAGAGT	Q-PCR primer for <i>spoIIE</i>
<i>spoIIE</i> -R	CCAACCAAACCTCCCACAGCTGCTAA	Q-PCR primer for <i>spoIIE</i>

**Table S2.** Expression ratios<sup>a</sup> of sporulation-related genes in WT, FKO1, and FKO1-C C.

*acetobutylicum* strains.

	<i>spoIIAA</i> (CAC2308)	<i>spoIIAB</i> (CAC2307)	<i>spoIIIE</i> (CAC3205)
Hour	FKO1/WT	FKO1/WT	FKO1/WT
12	0.05	0.05	0.21
24	0.33	0.48	1.91
36	0.40	0.54	1.04
Hour	FKO1-C/WT	FKO1-C/WT	FKO1-C/WT
12	1.12	1.17	0.70
24	1.10	1.11	0.38
36	0.83	0.83	0.15

<sup>a</sup> Cycle threshold ( $C_t$ ) values from two biological replicates (with three technical replicates each) were averaged together ( $n = 6$ ). Fold differences were calculated using the housekeeping gene CAC3571.