SUPPLEMENTAL TABLES AND FIGURES

Table S1 FAST gene sequence codon-optimized for *C. acetobutylicum*

ATGGAACACGTAGCATTTGGAAGTGAAGACATTGAAAACACTTTGGCAAAAATGGATGATGGAC
AGCTAGATGGTCTAGCATTCGGTGCTATACAGTTAGACGGTGACGGAAACATACTTCAGTATAA
CGCTGCTGAAGGTGATATAACAGGTAGAGACCCAAAGCAAGTAATTGGAAAAAATTTCTTTAAG
GACGTGGCTCCAGGTACTGATTCACCAGAGTTCTATGGAAAATTTAAGGAAGGAGTAGCTAGTG
GTAATTTGAATACAATGTTTGAATGGATGATACCAACAAGCAGGGGACCTACAAAAGTTAAAGT
ACACATGAAGAAAGCACTTTCTGGAGATAGCTACTGGGTTTTCGTTAAGAGGGTATGA

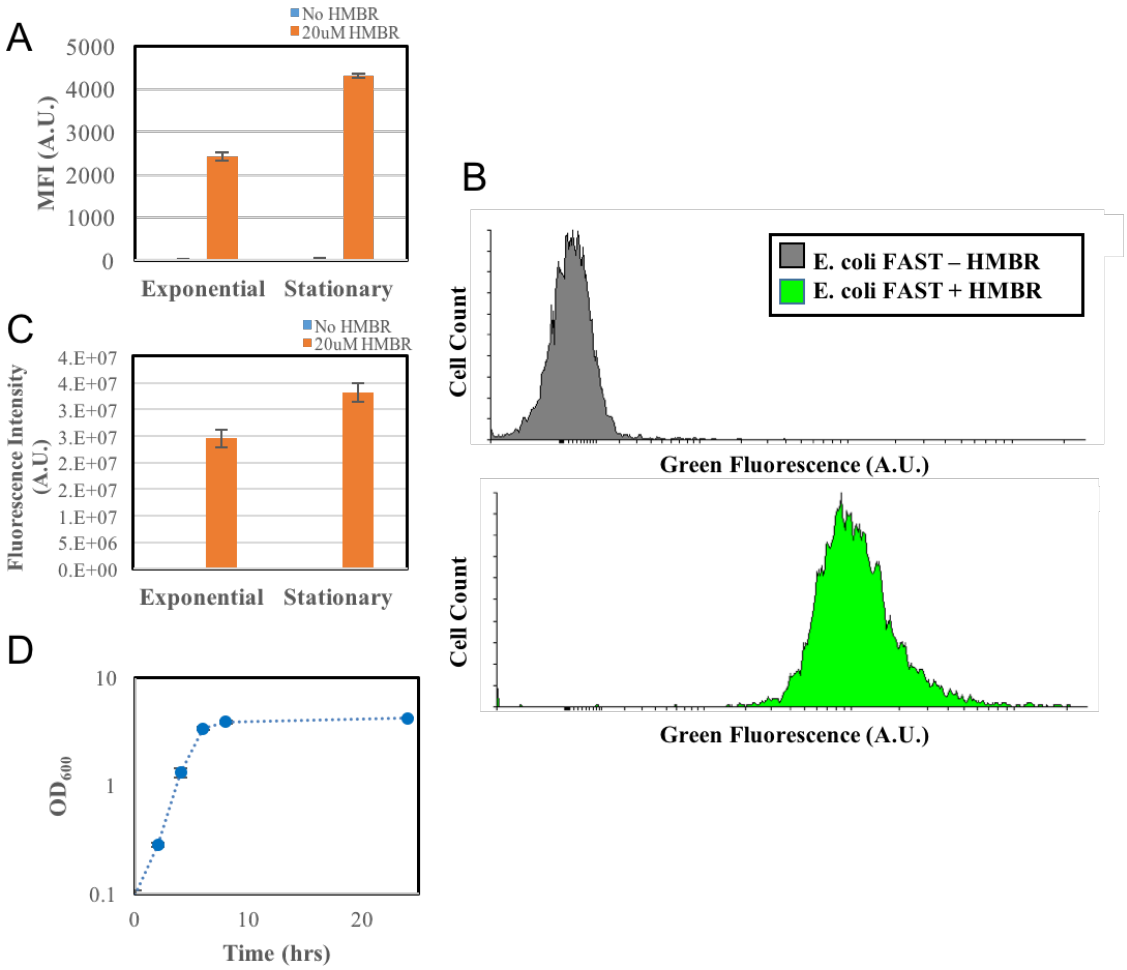

Figure S1 *E. coli* thlFAST shows promising fluorescence using flow cytometry and microplate reader. *A*, *E. coli* thlFAST MFI (A.U.) in LB medium + ampicillin after 4 hours (exponential) and 24 hours (stationary) using flow cytometry. *B*, Histograms showing *E. coli* thlFAST with No HMBR added (grey) and *E. coli* thlFAST with 20uM HMBR added

(green) after ~22 hours. *C*, *E. coli* thlFAST fluorescence intensity in LB medium + after 4 hours (exponential) and 24 hours (stationary) using SpectraMax i3x Microplate Reader. *D*, *E. coli* thlFAST growth curve in LB medium + ampicillin over 24 hours. n = 2, error bars: SD.

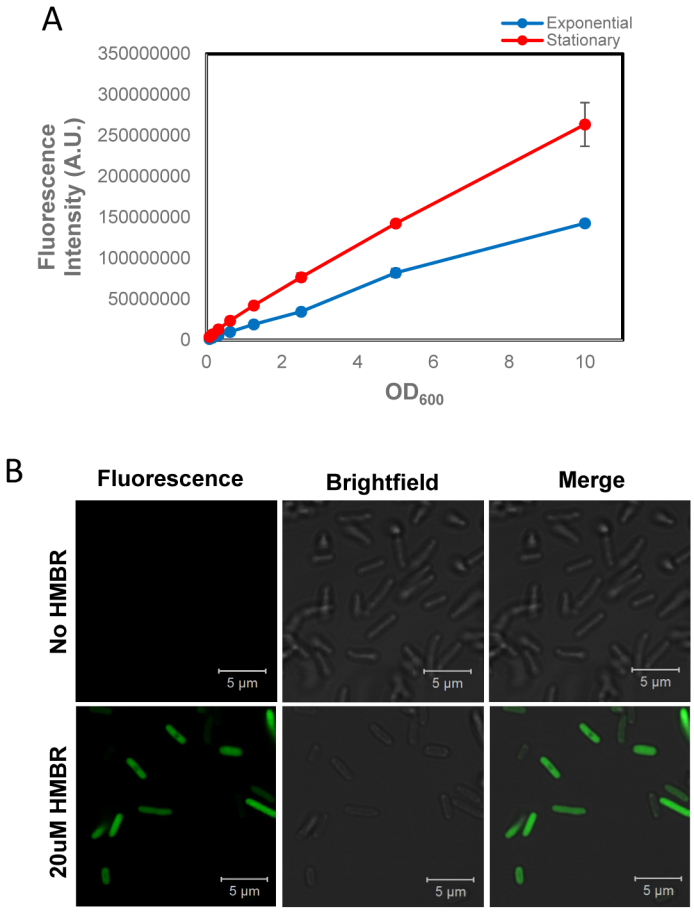

Figure S2 *E. coli* thlFAST shows stable linear fluorescence expression pattern in exponential and stationary phases of growth, shows higher fluorescence intensity in stationary phase of growth (red) compared to exponential phase of growth, and shows bright fluorescence using confocal microscopy. *A*, E. coli *thl*FAST fluorescence measured by microplate reader at 4 hours of growth (blue) and 24 hours of growth (red) resuspended in filtered PBS from OD_{600} 0.078 to OD_{600} 10. *B*, Confocal microscopy of

E. coli thlFAST with No HMBR (top) and 20uM HMBR (bottom), fluorescence (left) brightfield (middle) and merge (right). n = 2, error bars: SD.

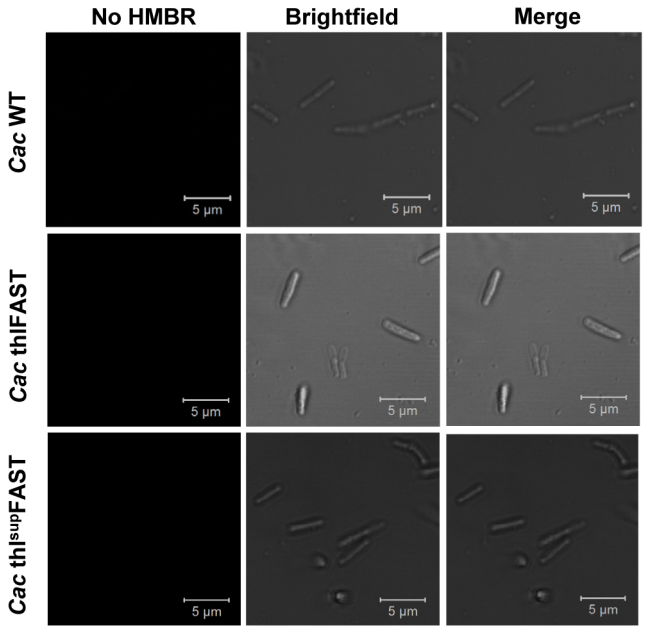

Figure S3 Confocal microscopy controls of *C. acetobutylicum* ATCC 824 (top), *C. acetobutylicum* thlFAST (middle), and *C. acetobutylicum* thl^{sup}FAST (bottom) with No HMBR (left), brightfield (middle), and merge (right) after ~30 hours of culture.

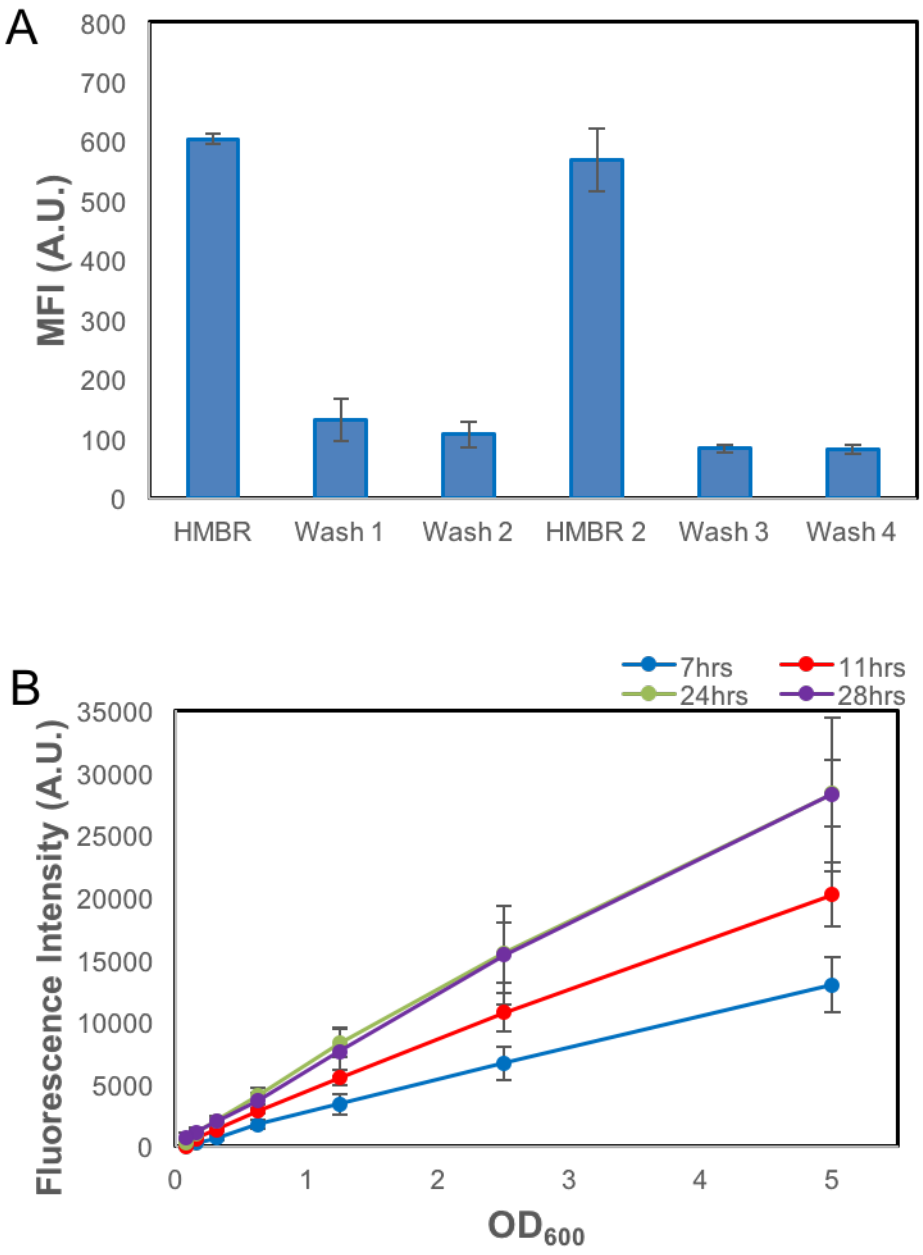

Figure S4 *C. acetobutylicum* thl^{sup}FAST shows HMBR binding reversibility and stable linear fluorescence over 28 hours. A, *C. acetobutylicum* thl^{sup}FAST was first added to 20 µM filtered HMBR in PBS (HMBR) and washed with filtered PBS (Wash 1). Cells were then washed a second time in filtered PBS (Wash 2). HMBR was then added to the cells again (HMBR 2) and washed again with filtered PBS twice (Wash 3, Wash 4).

Fluorescence was assessed by flow cytometry. B, C. acetobutylicum thl^{sup}FAST fluorescence measured by microplate reader at 7 hours (blue), 11 hours (red), 24 hours (green) and 28 hours of growth (purple) resuspended in filtered PBS with 20 uM HMBR from OD_{600} 0.078 to OD_{600} 5. n = 2 error bars: SD.

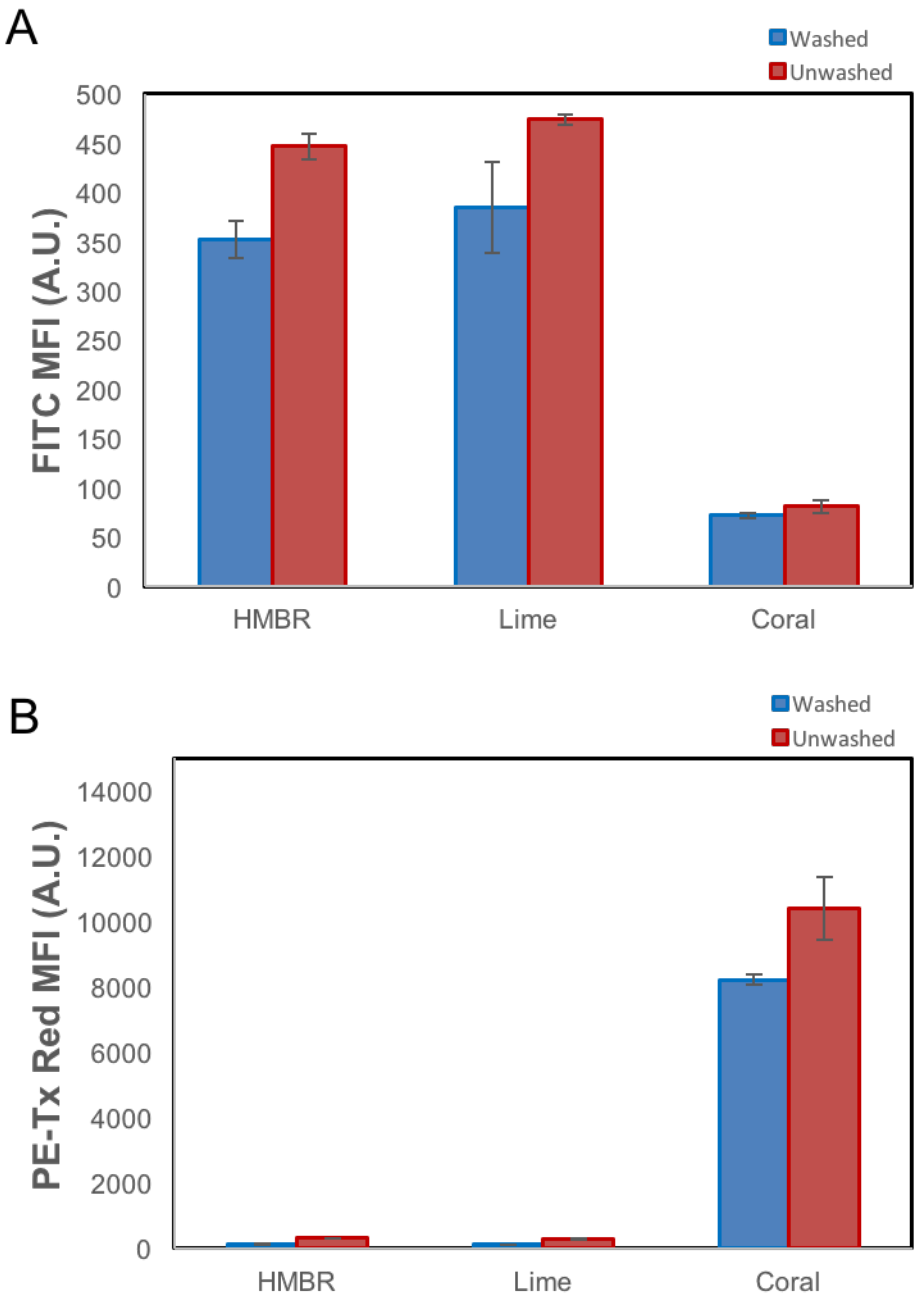

Figure S5 Our synthesized HMBR is comparable to commercially available HMBR (marketed as ^{TF}Lime); ^{TF}Coral fluorescence shows low overlap in emission with HMBR/ ^{TF}Lime using the PE-Tx Red filter. *A*, Filtered PBS washed (blue) and unwashed (red) *C. acetobutylicum* thl^{sup}FAST analyzed using the FITC fluorescence filter using synthesized HMBR (left), commercially available ^{TF}Lime (middle) and ^{TF}Coral (right). B,

Filtered PBS washed (blue) and unwashed (red) *C. acetobutylicum* thl^{sup}FAST analyzed using the PE-Tx Red fluorescence filter using synthesized HMBR (left), commercially available ^{TF}Lime (middle) and ^{TF}Coral (right). n = 2 error bars: SD.

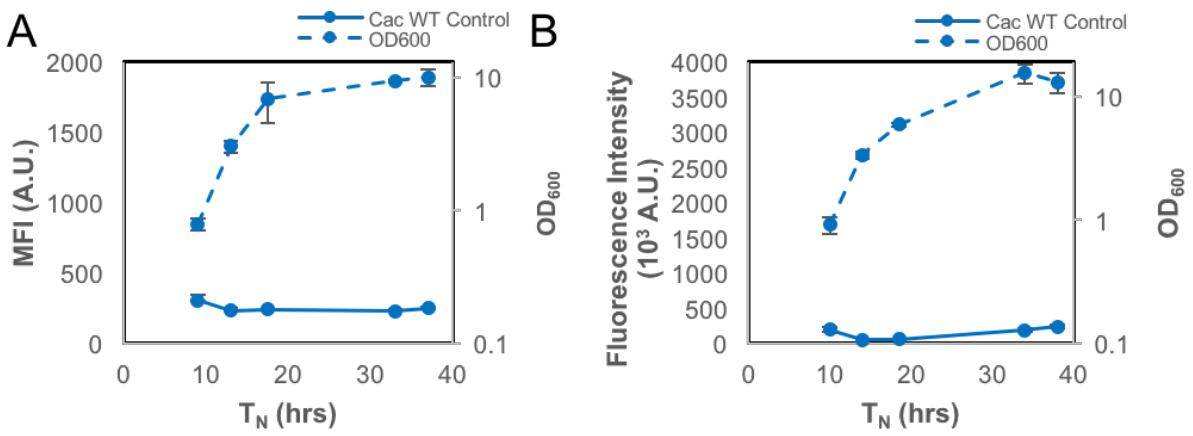

Figure S6 Flow cytometry and microplate reader controls of *C. acetobutylicum* ATCC 824 20uM HMBR. *A*, Geometric mean fluorescence intensity of *C. acetobutylicum* ATCC 824 with 20uM HMBR over ~37 hours using flow cytometry. *B*, Fluorescence intensity of *C. acetobutylicum* ATCC 824 over ~38 hours using microplate reader. n = 3, error bars: SD. Lag times were standardized between fermentations by normalizing an OD_{600} of 1.0 at hour 10 of growth as previously described (1).

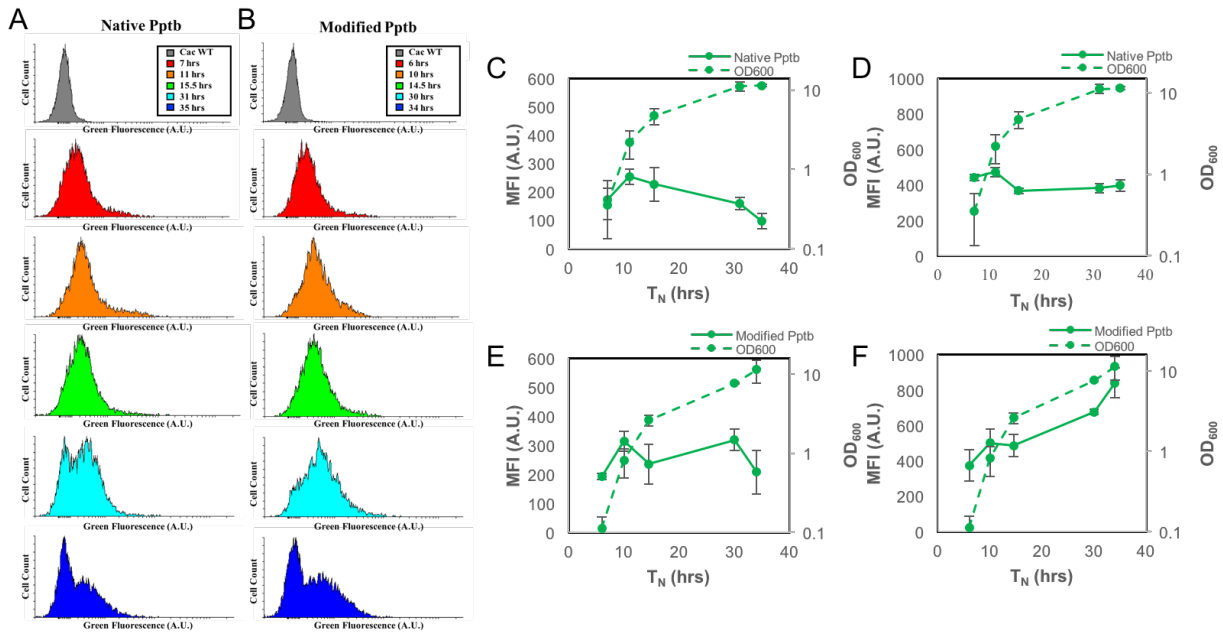

Figure S7 Histograms of ptbFAST and ptb^{mod}FAST over time show little difference in fluorescence shift over time, and comparing fluorescence of whole fluorescent population shows a promoter pattern similar to pattern shown by microplate reader. *A*, Histograms showing *C. acetobutylicum* ptbFAST (left column) after 7 hours (red), 11 hours (orange), 15.5 hours (green), 31 hours (light blue) and 35 hours (dark blue). *B*, Histograms showing *C. acetobutylicum* ptb^{mod}FAST (right column) after 6 hours (red), 10 hours (orange), 14.5 hours (green), 30 hours (light blue) and 34 hours (dark blue), compared with *C. acetobutylicum* WT (grey) after 9 hours of growth. *C*, *C. acetobutylicum* ptbFAST geometric means of entire population and growth curve over ~35 hours. n = 3. *D*, *C. acetobutylicum* ptbFAST geometric mean fluorescence of fluorescent population and growth curve over ~35 hours in Turbo CGM + erythromycin using using flow cytometry. n = 3. *E, C. acetobutylicum* ptb^{mod}FAST geometric means of entire population and growth curve over ~34 hours. n = 2. *F*, *C. acetobutylicum* ptb^{mod}FAST geometric mean fluorescence of the fluorescent population and growth

curve over ~34 hours in Turbo CGM + erythromycin using flow cytometry. n = 2 error bars: SD. Lag times were standardized between fermentations by normalizing an OD_{600} of 1.0 at hour 10 of growth as previously described (1).

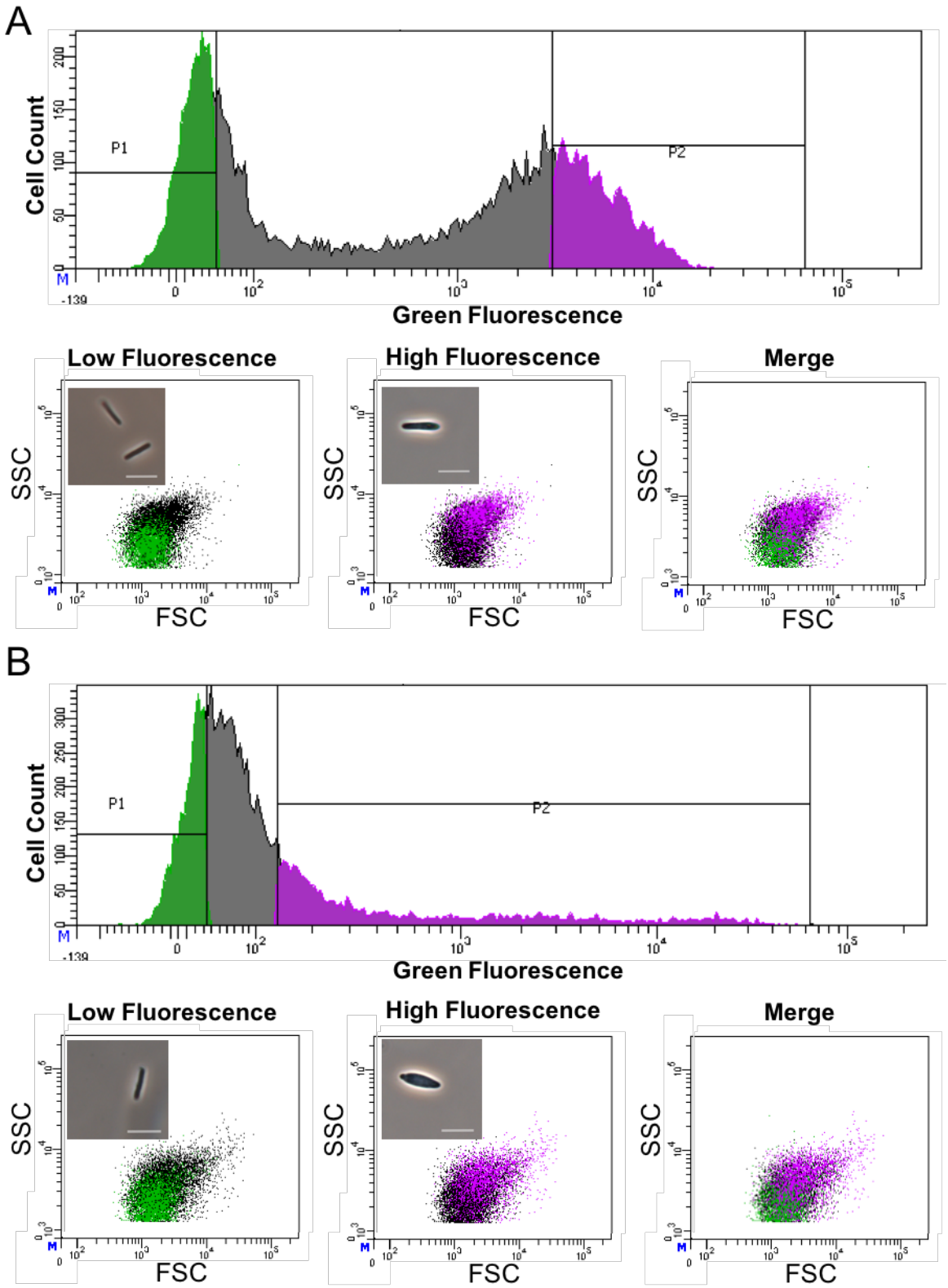
A
Cell Count
P1
P2
Green Fluorescence
Low Fluorescence
High Fluorescence
Merge
SSC
FSC
B
Cell Count
P1
P2
Green Fluorescence
Low Fluorescence
High Fluorescence
Merge
SSC
FSC

Figure S8 Sorting low (25%) and high (25%) fluorescence populations of *C. acetobutylicum* adcFAST shows that FAST is expressed in both vegetative and clostridial form morphologies and low fluorescence cells remain in a vegetative state. *A*, *C. acetobutylicum* adcFAST histogram (top panel) of low fluorescence (P1) and high fluorescence (P2) and dot plots (bottom panel) with accompanying phase contrast images of sorted cell populations after 32 hours of growth. *B*, *C. acetobutylicum* adcFAST histogram (top panel) of low fluorescence (P1) and high fluorescence (P2) and dot plots (bottom panel) with accompanying phase contrast images of sorted cell populations after 56 hours of growth. White scale bar in picture inserts represent 5 μm.

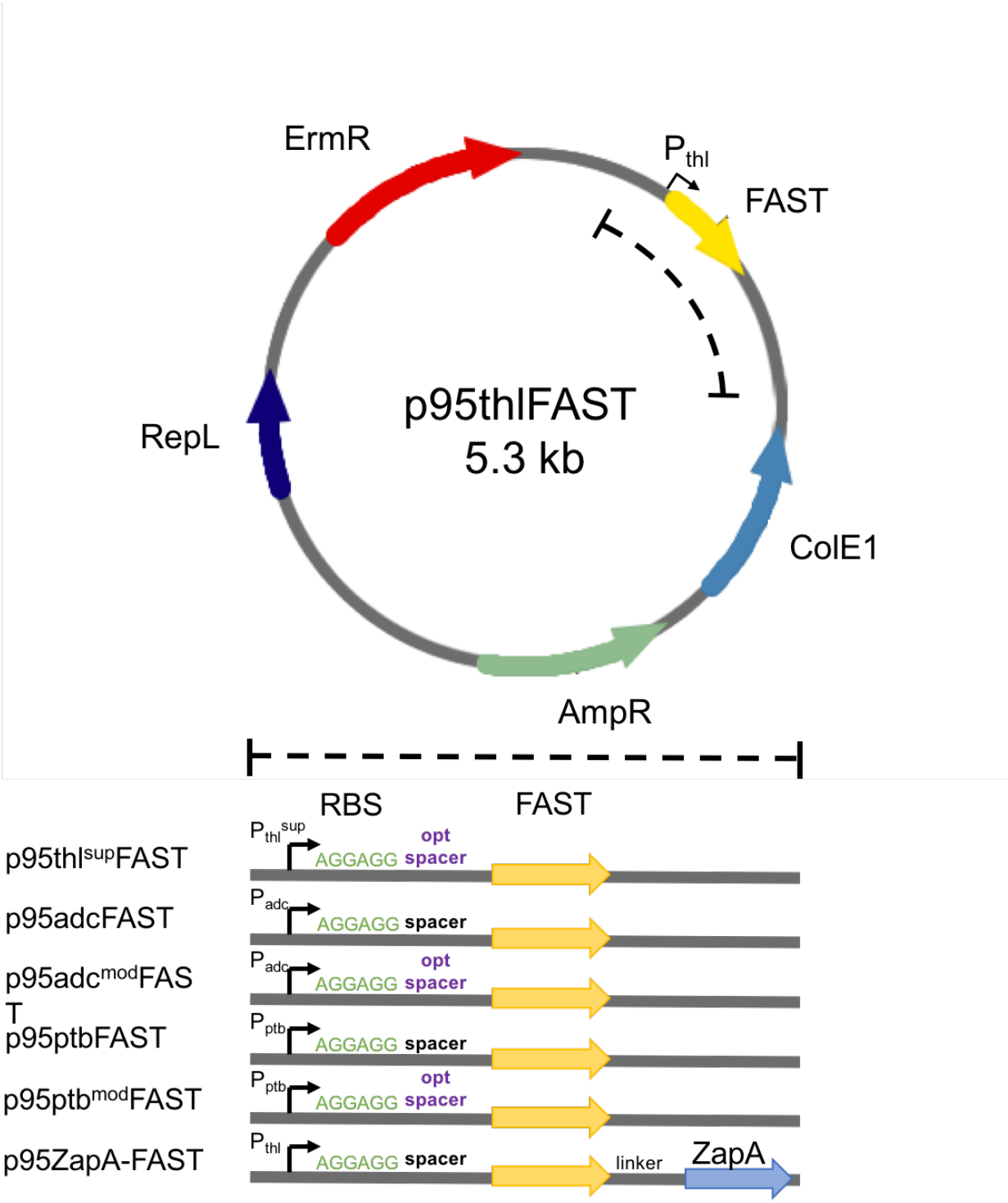

Figure S9 A schematic of the genetic organization of the p95thlFAST reporter plasmid and organization of each reporter region of p95thlsupFAST, p95adcFAST, p95adcmodFAST, p95ptbFAST, p95ptbmodFAST and of p95ZapA-FAST fusion protein.

p95thlFAST *E. coli – C. acetobutylicum* shuttle vector shows the Gram-negative origin of replication (ColE1), Gram-positive origin of replication (RepL), Ampicillin resistance (AmpR), Erythromycin resistance (ErmR) and FAST coding regions. The dashed line indicates the area of the plasmid that varies between plasmids. Each reporter region shows the promoter, ribosome binding site, the type of spacer, and gene of interest. opt spacer: optimized spacer from P_{thl} 1200-9-9 (P_{thl}^{sup})(2): nucleic acid sequence - TTAGGATCC; spacer: native spacer; linker: protein sequence - GGGS.

References:

1. Sillers R, Al-Hinai MA, Papoutsakis ET. 2009. Aldehyde-Alcohol Dehydrogenase and/or Thiolase Overexpression Coupled With CoA Transferase Downregulation Lead to Higher Alcohol Titers and Selectivity in Clostridium acetobutylicum Fermentations. Biotechnol Bioeng 102:38-49.
2. Yang G, Jia D, Jin L, Jiang Y, Wang Y, Jiang W, Gu Y. 2017. Rapid Generation of Universal Synthetic Promoters for Controlled Gene Expression in Both Gas-Fermenting and Saccharolytic Clostridium Species. ACS Synth Biol 6:1672-1678.