Supplementary Information for

Identification of Transport Proteins Involved in Free Fatty Acid Efflux in *Escherichia coli*

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Table S1: List of oligonucleotide primers used in this study.

	Primer name[a]	Sequence (5' to 3')[b]
1	fadD_colPCR_fwd	ACGGCATGTATATCATTTGGG
2	fadD_colPCR_rev	CTTTAGTGGGCGTCAAAAAAAAC
3	araBAD_colPCR_fwd	AAGCGGGACCAAAGCCATGAC
4	araBAD_colPCR_rev	AGGAGACTTCTGTCCCTTGCG
5	araFGH_colPCR_fwd	GGTACCAAAGACAACAAGGATTTCC
6	araFGH_colPCR_rev	CTATACTTACATGTCTGTAAAGCGCG
7	ParaE_colPCR_fwd	CATGGCGACCAACAATACTC
8	ParaE_colPCR_rev	TTCCGCCTCAATATGACG
9	fadE_colPCR_fwd	CGATTGATGGTAAAACGGTGTTGTT
10	fadE_colPCR_rev	CTGAAGTGCGGATAAAAACAGCAA
11	fadAB_colPCR_fwd	GGAGTGAATAAGTAACGCATCC
12	fadAB_colPCR_rev	GCTGTCGCGTCTTATCGTGC
13	acrAB_KO_fwd	ACCATTGACCAATTTGAAATCGGACACTCGAGGTTTACATGTGT AGGCTGGAGCTGCTTC
14	acrAB_KO_rev	CCGCTTACGCGGCCTTAGTGATTACACGTTGTATCAATTCCGGG GATCCGTCGACC
15	mdtEF_KO_fwd	TTAAAGAACCGTTATTTCTCAAGAATTTTCAGGGACTAAAGTGT AGGCTGGAGCTGCTTC
16	mdtEF_KO_rev	CTGAACCTTCATGTTCAACCTTACTCTCATTTACACGTTAATTC CGGGGATCCGTCGACC
17	acrEF_KO_fwd	TTGGGTAAATAACGCGCTTTTGGTTTTTTGAGGAATAGTAGTGT AGGCTGGAGCTGCTTC
18	acrEF_KO_rev	ATATAAAGGCACCCGAAAGCGCCTTTATGTTTCTGATTTAATTC CGGGGATCCGTCGACC
19	emrAB_KO_fwd	TCGGCTCAGCCGATGAGTTAAGAAGATCGTGGAGAACAATGTGT AGGCTGGAGCTGCTTC
20	emrAB_KO_rev	TGAACTGGCTTAGTTGTACTTAGTGCGCACCGCCTCCGCCATTC CGGGGATCCGTCGACC
21	mdtABCD_KO_fwd	ATTCCGCGAAACGTTTCAGGAAGAGAAACTCTTAACGATGGTGT AGGCTGGAGCTGCTTC
22	mdtABCD_KO_rev	GTAATACCGGGTCGCCAGAACTTCATTGCGCGCTCCTTTTATTC CGGGGATCCGTCGACC
23	rob_colPCR_fwd	GTCAAGCCCTAAAACATACTCTAC
24	rob_colPCR_rev	GATGCCTGGTGAGTACGATTC
25	tolC_colPCR_fwd	ACGCCAACCTTTTGCGGTAG
26	tolC_colPCR_rev	GAAGAATGCGGCAGATAACC
27	fadL_colPCR_fwd	GCCACTGGTCTGATTTCTAAG
28	fadL_colPCR_rev	CAAAACTCAGGAGGTAAGCAATG
29	prc_colPCR_fwd	AGTCTGGATAGTGCGTAAGTC
30	prc_colPCR_rev	GCTGAATTCGGGTATGTCTTTG

Table S1 (cont.)

	Primer name[a]	Sequence (5' to 3')[b]
31	acrD_colPCR_fwd	TCGTACCTTGCCGCTACAGTG
32	acrD_colPCR_rev	CAAAGTACAAACAGCAAGAACCCG
33	mdtG_colPCR_fwd	GATAAAAGCTCTCTGGATTGCG
34	mdtG_colPCR_rev	GCAGCGTCAATGGCTTCTTC
35	mdtK_colPCR_fwd	GAATGGCTATTTTTTCACTGGAG
36	mdtK_colPCR_rev	GATAACAGATGCCAGTCGG
37	cmr_colPCR_fwd	GTAGCTATACTCGTAATAATGTAAG
38	cmr_colPCR_rev	CCTTATGTTCGCCATCTTGC
39	ompF_colPCR_fwd	AGACACCAAACTCTCATCAATAGTTC
40	ompF_colPCR_rev	AACGCAGGCTGTTTTTGCAAGAC
41	acrAB_colPCR_fwd	CAGGCGTTAGATTTACATACATTTG
42	acrAB_colPCR_rev	CCGTGGTTAATACTGGTTTTCG
43	mdtEF_colPCR_fwd	GTGCCTGTATCCCACCTTAC
44	mdtEF_colPCR_rev	GATGACGAATGGCTGGAGTG
45	acrEF_colPCR_fwd	CCCGCGTCAAAATAAAACAGTAG
46	acrEF_colPCR_rev	CGGAGGTTATAAATCTTGCGG
47	emrAB_colPCR_fwd	CTCCCGTCTCGACCAGATGG
48	emrAB_colPCR_rev	CTCGTTGCAGGAAGCGCAGG
49	mdtABCD_colPCR_fwd	CATTCAGACGATTCCAGACA
50	mdtABCD_colPCR_rev	AGCCACGCTCAAAACTGATAC
51	pBAD33-C280*_fwd	GGGCTCGAGTTAACCGGCACGGAACTCGCTCG
52	pBAD33-C280*_rev	GGGCTCGAGTTGGTAACGAATCAGACAATTGACGGC
53	acrAB_fwd	TTTTCCCGGGCACTCGAGGTTTACATATGAAC
54	acrAB_rev	CCCTCTAGATCAATGATGATCGACAGTATGG
55	tolC_fwd	GGGTCTAGATTCACCACAAGGAATGCAAATGAAGAAATTGCTCC CCATTCTTATC
56	tolC_rev	GGGGCATGCTCAGTTACGGAAAGGGTTATGACC
57	emrAB_fwd	GCCCCCGGGTCGTGGAGAACAATATGAGCGCAAATG
58	emrAB_rev	GGGTCTAGATTAGTGCGCACCGCCTCCG
59	mdtEF_fwd	GGGGAGCTCAAGAATTTTCAGGGACTAAAA
60	mdtEF_rev	AAACCCGGGCATTTACACGTTACGCTTTTT
61	mdtABCD_fwd	AAAGAGCTCCAGGAAGAGAAACTCTTAACGATG
62	mdtABCD_rev	AAACCCGGGACTTCATTGCGCGCTCCTTTT

[a] Primers containing 'colPCR' were used colony PCR verification of chromosomal gene insertions and deletions.
Primers containing restriction sites were used for amplification of insertions for cloning.
Primers containing 'KO' were used for generating linear cassettes for homologous recombination in strain DY330.
[b] Restriction sites are underlined

Table S2: Full table of bacterial strains and plasmids used in this study.

Strain	Relevant genotype/property[a]	Source or reference
K-12 MG1655	F^- λ^- *ilvG*$^-$ *rfb*-50 *rph*-1	CGSC
DH5α	*fhuA2* Δ(*argF-lacZ*)*U169 phoA glnV44* Φ*80* Δ(*lacZ*)*M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Invitrogen
TY05	K-12 MG1655 *fadD*::P_{trc}-BTE *fadE*::P_{trc}-BTE *fadAB*::P_{trc}-BTE	(9)
TY06	K-12 MG1655 *fadD*::P_{trc}-BTE-H204A *fadE*::P_{trc}-BTE-H204A *fadAB*::P_{trc}-BTE-H204A	(9)
TY05ara	K-12 MG1655 *fadD*::P_{trc}-BTE *fadE*::P_{trc}-BTE *fadAB*::P_{trc}-BTE Δ*araFGH* Φ(Δ*araEp* P_{CP18}-*araE*) Δ*araBAD*	This work
BW25113	*lacI*^{q} *rrnB3* F- *Δ(araD-araB)567 ΔlacZ4787*(::rrnB-3) λ^- *rph-1 Δ(rhaD-rhaB)568 hsdR514*	(1)
JW5249-1	BW25113 Δ*marA752::kan*	(1)
JW4359-1	BW25113 Δ*rob-721::kan*	(1)
JW4023-5	BW25113 Δ*soxS756::kan*	(1)
JW5503-1	BW25113 Δ*tolC732::kan*	(1)
JW2341-1	BW25113 Δ*fadL752*::*kan*	(1)
JW1819-1	BW25113 Δ*prc-755*::*kan*	(1)
JW2454-1	BW25113 Δ*acrD790*::*kan*	(1)
JW1040-1	BW25113 Δ*mdtG723::kan*	(1)
JW1655-1	BW25113 Δ*mdtK740::kan*	(1)
JW0826-1	BW25113 Δ*cmr-742::kan*	(1)
JW0912-1	BW25113 Δ*ompF746::kan*	(1)
DY330	K-12 W3110 Δ*lacU169 gal490 pgl*Δ8 λ*c*I*857* Δ(*cro-bioA*) (Tet^R)	(8)
BW27269	BW25113 *araFGH*::*kan903*	(4)
BW27270	BW25113 Φ(Δ*araEp kan* P_{CP18}-*araE*)	(4)
RL36	K-12 MG1655 *fadD*::P_{trc}-BTE *fadE*::P_{trc}-BTE *fadAB*::P_{trc}-BTE *araFGH*::*kan*	This work
RL37	K-12 MG1655 *fadD*::P_{trc}-BTE *fadE*::P_{trc}-BTE *fadAB*::P_{trc}-BTE Δ*araFGH*	This work
RL38	K-12 MG1655 *fadD*::P_{trc}-BTE *fadE*::P_{trc}-BTE *fadAB*::P_{trc}-BTE Δ*araFGH* Φ(Δ*araEp kan* P_{CP18}-*araE*)	This work
RL39	K-12 MG1655 *fadD*::P_{trc}-BTE *fadE*::P_{trc}-BTE *fadAB*::P_{trc}-BTE Δ*araFGH* Φ(Δ*araEp* P_{CP18}-*araE*)	This work
RL40	K-12 MG1655 *fadD*::P_{trc}-BTE *fadE*::P_{trc}-BTE *fadAB*::P_{trc}-BTE Δ*araFGH* Φ(Δ*araEp* P_{CP18}-*araE*) *araBAD*::*cat*	This work

Table S2 (cont.)

Strain	Relevant genotype/property[a]	Source or reference
RL46	DY330 *acrAB*::*kan*	This work
RL47	DY330 *mdtEF*::*kan*	This work
RL48	DY330 *acrEF*::*kan*	This work
RL49	DY330 *emrAB*::*kan*	This work
RL50	DY330 *mdtABCD*::*kan*	This work
RL51	TY05 *marA::kan*	This work
RL52	TY06 *marA::kan*	This work
RL53	TY05 *rob::kan*	This work
RL54	TY06 *rob::kan*	This work
RL55	TY05 *soxS::kan*	This work
RL56	TY06 *soxS::kan*	This work
RL57	TY05 *tolC::kan*	This work
RL58	TY06 *tolC::kan*	This work
RL59	TY05 *fadL::kan*	This work
RL60	TY06 *fadL::kan*	This work
RL61	TY05 *prc::kan*	This work
RL62	TY06 *prc::kan*	This work
RL63	TY05 *acrD::kan*	This work
RL64	TY06 *acrD::kan*	This work
RL65	TY05 *mdtG::kan*	This work
RL66	TY06 *mdtG::kan*	This work
RL67	TY05 *mdtK::kan*	This work
RL68	TY06 *mdtK::kan*	This work
RL69	TY05 *cmr::kan*	This work
RL70	TY06 *cmr::kan*	This work
RL71	TY05 *ompF::kan*	This work
RL72	TY06 *ompF::kan*	This work
RL73	TY05 *acrAB::kan*	This work
RL74	TY06 *acrAB::kan*	This work
RL75	TY05 *mdtEF::kan*	This work
RL76	TY06 *mdtEF::kan*	This work
RL77	TY05 *acrEF::kan*	This work
RL78	TY06 *acrEF::kan*	This work
RL79	TY05 *emrAB::kan*	This work
RL80	TY06 *emrAB::kan*	This work
RL81	TY05 *mdtABCD::kan*	This work
RL82	TY06 *mdtABCD::kan*	This work
RL83	TY05 Δ*marA*	This work
RL84	TY06 Δ*marA*	This work
RL85	TY05 Δ*rob*	This work
RL86	TY06 Δ*rob*	This work

Table S2 (cont.)

Strain	Relevant genotype/property[a]	Source or reference
RL87	TY05 Δ*soxS*	This work
RL88	TY06 Δ*soxS*	This work
RL89	TY05 Δ*tolC*	This work
RL90	TY06 Δ*tolC*	This work
RL91	TY05Δ*fadL*	This work
RL92	TY06 Δ*fadL*	This work
RL93	TY05 Δ*prc*	This work
RL94	TY06 Δ*prc*	This work
RL95	TY05 Δ*acrD*	This work
RL96	TY06 Δ*acrD*	This work
RL97	TY05 Δ*mdtG*	This work
RL98	TY06 Δ*mdtG*	This work
RL99	TY05 Δ*mdtK*	This work
RL100	TY06 Δ*mdtK*	This work
RL101	TY05 Δ*cmr*	This work
RL102	TY06 Δ*cmr*	This work
RL103	TY05 Δ*ompF*	This work
RL104	TY06 Δ*ompF*	This work
RL105	TY05 Δ*acrAB*	This work
RL106	TY06 Δ*acrAB*	This work
RL107	TY05 Δ*mdtEF*	This work
RL108	TY06 Δ*mdtEF*	This work
RL109	TY05 Δ*acrEF*	This work
RL110	TY06 Δ*acrEF*	This work
RL111	TY05 Δ*emrAB*	This work
RL112	TY06 Δ*emrAB*	This work
RL113	TY05 Δ*mdtABCD*	This work
RL114	TY06 Δ*mdtABCD*	This work
RL115	TY05 Δ*acrAB acrD::kan*	This work
RL116	TY06 Δ*acrAB acrD::kan*	This work
RL117	TY05 Δ*acrAB mdtEF::kan*	This work
RL118	TY06 Δ*acrAB mdtEF::kan*	This work
RL119	TY05 Δ*acrAB acrEF::kan*	This work
RL120	TY06 Δ*acrAB acrEF::kan*	This work
RL121	TY05 Δ*acrAB emrAB::kan*	This work
RL122	TY06 Δ*acrAB emrAB::kan*	This work
RL123	TY05 Δ*acrAB mdtABCD::kan*	This work
RL124	TY06 Δ*acrAB mdtABCD::kan*	This work
RL125	TY05 Δ*acrAB* Δ*acrD*	This work
RL126	TY06 Δ*acrAB* Δ*acrD*	This work
RL127	TY05 Δ*acrAB* Δ*mdtEF*	This work

Table S2 (cont.)

Strain	Relevant genotype/property[a]	Source or reference
RL128	TY06 Δ*acrAB* Δ*mdtEF*	This work
RL129	TY05 Δ*acrAB* Δ*acrEF*	This work
RL130	TY06 Δ*acrAB* Δ*acrEF*	This work
RL131	TY05 Δ*acrAB* Δ*emrAB*	This work
RL132	TY06 Δ*acrAB* Δ*emrAB*	This work
RL133	TY05 Δ*acrAB* Δ*mdtABCD*	This work
RL134	TY06 Δ*acrAB* Δ*mdtABCD*	This work
RL135	TY05ara *acrAB::kan*	This work
Plasmids		
pKD13	Template plasmid, R6K gamma origin, Amp^R, Kan^R	(2)
pCP20	carries yeast FLP recombinase under constitutive promoter, pSC101 origin, λ $cI857^+$, λ p_R Rep^{ts}, Amp^R, Cm^R	(2)
pBAD33	P_{BAD} promoter, pACYC origin, Cm^R	(3)
pBAD33-C280*	pBAD33 with *araC*-C280* mutation	This work
pBAD33-C280*-tolC	pBAD33-C280* carrying *tolC* under P_{BAD} control, Cm^R	This work
pBAD33-C280*-acrAB	pBAD33-C280* carrying *acrAB* under P_{BAD} control, Cm^R	This work
pBAD33-C280*-acrAB-tolC	pBAD33-C280* carrying *acrAB-tolC* artificial operon under P_{BAD} control, Cm^R	This work
pBAD33-C280*-mdtEF	pBAD33-C280* carrying *mdtEF* under P_{BAD} control, Cm^R	This work
pBAD33-C280*-mdtEF-tolC	pBAD33-C280* carrying *mdtEF-tolC* artificial operon under P_{BAD} control, Cm^R	This work
pBAD33-C280*-emrAB	pBAD33-C280* carrying *emrAB* under P_{BAD} control, Cm^R	This work
pBAD33-C280*-emrAB-tolC	pBAD33-C280* carrying *emrAB-tolC* artificial operon under P_{BAD} control, Cm^R	This work
pBAD33-C280*-mdtABCD	pBAD33-C280* carrying *mdtABCD* under P_{BAD} control, Cm^R	This work
pBAD33-C280*-mdtABCD-tolC	pBAD33-C280* carrying *mdtABCD-tolC* artificial operon under P_{BAD} control, Cm^R	This work

[a]Abbreviations: Amp, ampicillin; Cm, chloramphenicol; Kan, kanamycin; R, resistance; ts, temperature sensitive.

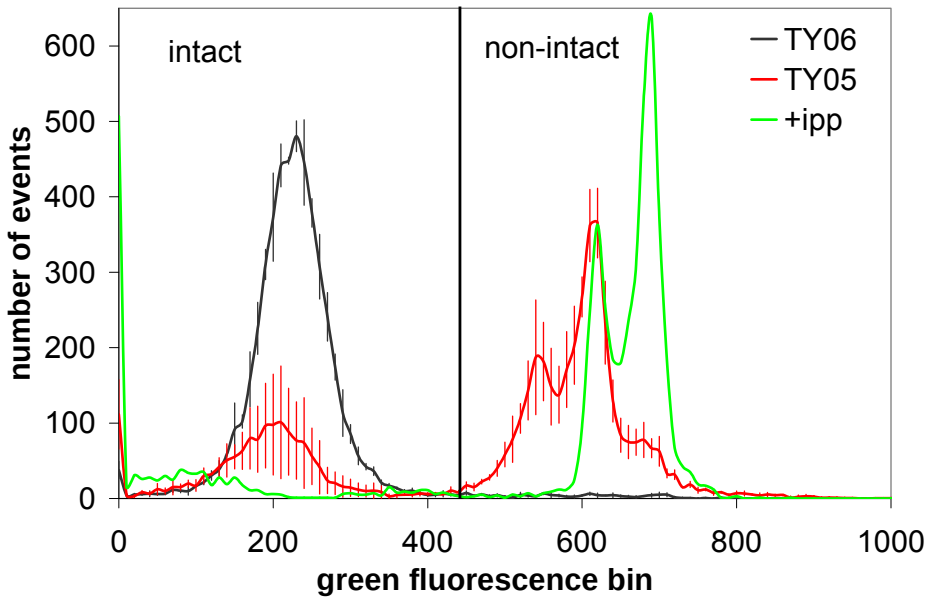

Figure S1: Determination of threshold for non-intact SYTOX Green stained cells from green fluorescence histograms. Induced cultures of TY06 and TY05 were stained after 8 h growth, and a TY05 strain (TY05/pBAD33*) was additionally treated with 25% v/v isopropanol (ipp, green curve) for 10 minutes, to demonstrate justification of a green fluorescence value of 440 as the threshold between intact and non-intact cells. Error bars represent standard deviations in each histogram bin from three biological replicate cultures.

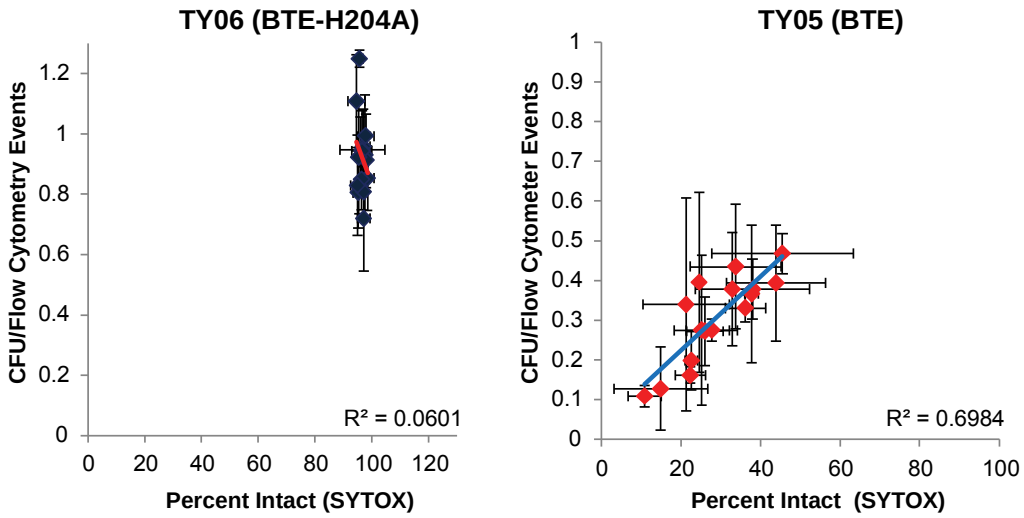

Figure S2: Scatter plots of normalized CFUs versus percent intact cells by SYTOX Green staining. (Left) TY06 background strain data points with linear fit having R^2 value of 0.0601. (Right) TY05 background strain data points with linear fit having R^2 value of 0.6984.

Supplementary Results 1: MIC of exogenous FFAs in single gene/operon deletion strains

To further confirm the role of the identified genes in conferring resistance to free fatty acids, deletion strains in TY05 were plated under non-inducing conditions (no added IPTG) on LB agar containing varying concentrations of octanoic and decanoic acid. The pH was adjusted to 7 in all plates by addition of equimolar amounts of NaOH, and it was confirmed that the maximum concentration of Na^+ present was not growth inhibitory toward TY05 or TY05 Δ*acrAB* in a plate containing NaCl (data not shown). Dodecanoic and tetradecanoic acids were non-inhibitory at plate concentrations of 2 g/L, which was also above their solubility limits. We have previously observed minimal toxicity of 0.5 g/L dodecanoic acid added to cultures and have postulated that endogenously produced dodecanoic acid, or the mixture of fatty acids resulting from expression of BTE, exhibits a higher degree of toxicity (5). In contrast, sodium octanoate and decanoate appear soluble at concentrations of up to 10 g/L and 5 g/L, respectively, and both elicit growth inhibition in strain TY05 at concentrations below these apparent solubilities. Saturated overnight cultures of TY05, TY05 Δ*acrAB*, TY05 Δ*emrAB*, TY05 Δ*mdtEF*, TY05 Δ*mdtABCD*, TY05 Δ*tolC*, and TY05 Δ*rob* were diluted 1:100 in 5 mL of LB agar in glass tubes and incubated at 37°C for 4 h with 250 rpm shaking. At this time, all cultures had an OD_{600} between 2.5 to 2.9. Samples from each culture were serially diluted in PBS and 3 μL of 10^4, 10^5, and 10^6-fold dilutions were spotted on plates containing varying concentrations of octanoate and decanoate (**Figure S3**). After overnight incubation at 37°C, growth of TY05 was observed up to 5 g/L octanoate and 4 g/L decanoate. Growth was observed for TY05 Δ*acrAB* only for 0 g/L decanoate (no growth at 0.5 g/L) and up to 3 g/L octanoate, while TY05 Δ*rob* grew on up to 3 g/L decanoate and 4 g/L octanoate. Growth was observed of TY05 Δ*mdtABCD* up to 4 g/L decanoate (same as TY05) but only up to 4 g/L octanoate. TY05 Δ*tolC* was the most inhibited,

showing greatly reduced growth on plates containing 0 g/L octanoate and no growth on 0 g/L decanoate, despite the similar OD_{600} to all other strains grown in liquid LB medium. Presumably, the presence of either Brij-35 or ethanol was responsible for the inhibition of growth on plates. However, while some growth was observed for TY05 Δ*tolC* on 0 g/L octanoate, no growth was observed for 1 g/L or any higher concentration, indicative of a fatty acid-specific effect. All other strains tested had similar MICs toward octanoate and decanoate as background strain TY05.

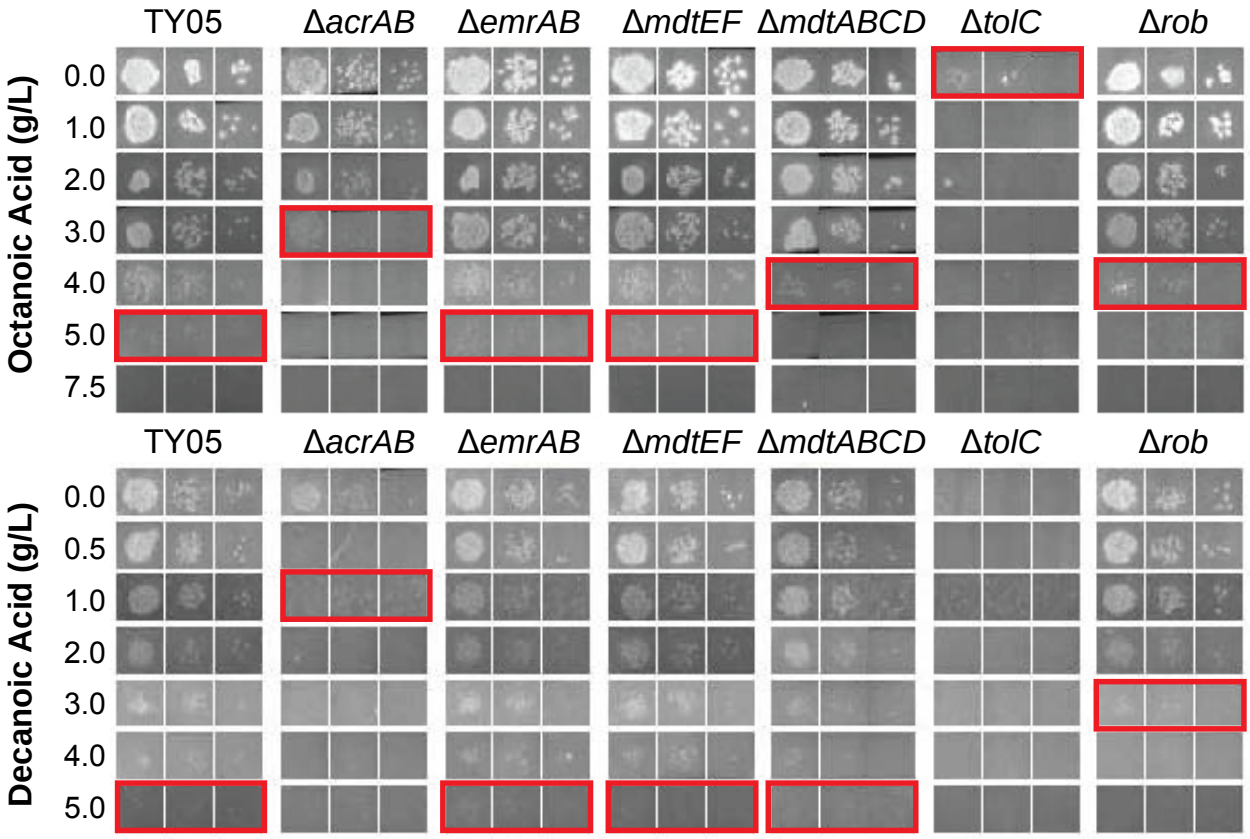

Figure S3: MIC assay for octanoate and decanoate against TY05 and selected single deletions in TY05. (top) TY05 exhibits visible growth up to 5 g/L octanoate. Deletions in *acrAB*, *rob*, *mdtABCD*, and *tolC* resulted in reduced MICs of 3, 4, 4, and 0 g/L octanoate, respectively. (bottom) TY05 exhibits visible growth up to 4 g/L decanoate. Deletions in *acrAB*, *rob*, and *tolC* resulted in reduced MICs of 0.5, 3 g/L decanoate, and no growth, respectively. Red boxes denote the maximum concentration at which growth was observed after incubation for one night at 37°C and several more days at room temperature.

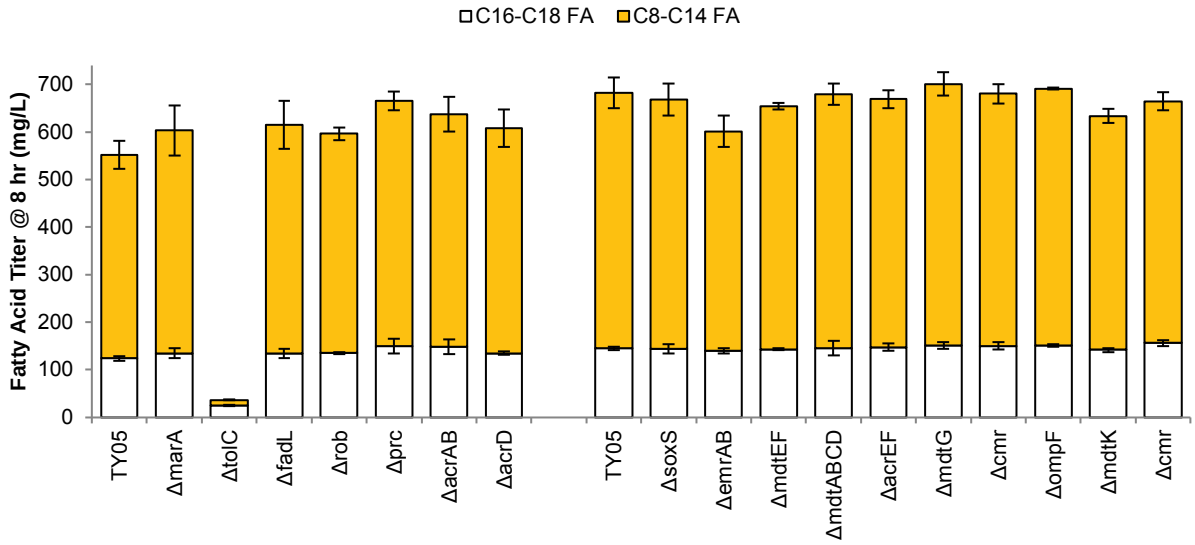

Figure S4: Total fatty acid titers in TY05 deletion strains after 8 h. Fatty acids were extracted from TY05 cultures grown for 8 h in LB + 0.4% glycerol in two separate experiments (the results of TY05 are shown for each experiment). In general no significant differences were observed except for TY05 Δ*tolC*.

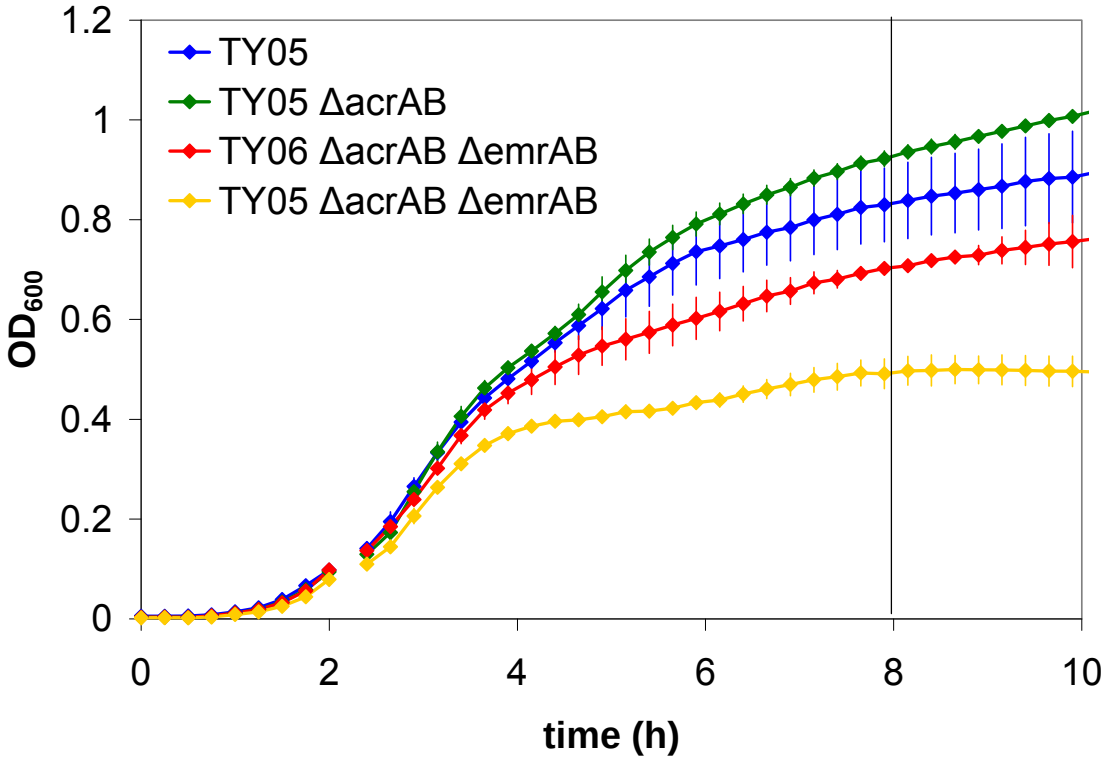

Figure S5: Plate reader growth curves of *acrAB emrAB* double deletion strains and negative control strains. Biological triplicate cultures were grown in 96-well plates in LB + 0.4% glycerol with shaking at 37°C and induced after 2 h with 1 mM IPTG. TY05 Δ*acrAB* Δ*emrAB* exhibited a reduced OD_{600} at 8 h (marked with vertical line), the sampling time at which CFU/mL and SYTOX Green staining was performed from shake flask cultures.

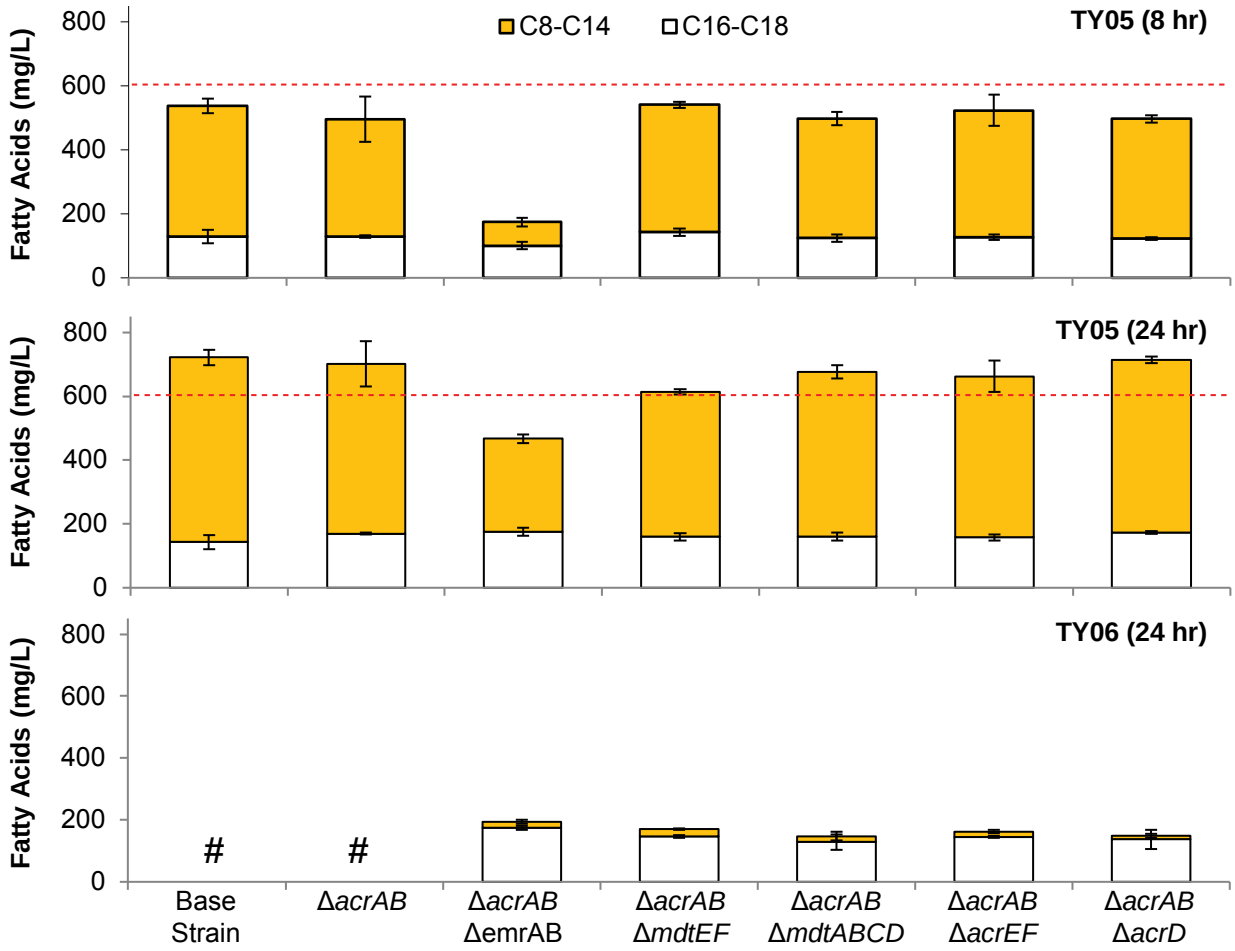

Figure S6: Total fatty acid titers for double efflux pump deletions in TY05 and TY06. Fatty acids were sampled 8 h and 24 h post-inoculation. TY05 Δ*acrAB* Δ*emrAB* exhibits greatly reduced fatty acid production (primarily reduced C_8-C_{14}) relative to other TY05 strains after 8 h. Low titers relative to other TY05 strains persist in TY05 Δ*acrAB* Δ*emrAB* after 24 h. # - not measured.

Supplementary Results 2: Functional validation of drug efflux pump expression constructs

All identified gene/operon deletions that exhibited reduced viabilities encode for TolC-associated multidrug efflux pumps that have previously been shown to confer resistance to SDS (6, 7). While wild-type *E. coli* exhibited an MIC toward SDS of greater than 12.8 mg/mL, deletions in *acrAB* reduced the MIC to between 0.05 and 0.1 mg/mL (6, 7). Expression of *acrAB*, *emrAB*, *mdtEF*, and *mdtABCD* on high copy plasmids in an *acrAB* deletion strain increased the MIC toward SDS from 0.05 mg/mL to greater than 0.4 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.2 mg/mL, respectively (6). Therefore an increase in MIC of SDS was used to validate the functional expression of multidrug efflux pumps cloned into pBAD33*. As previously observed, strain TY05 (with intact *acrAB*) harboring empty vector pBAD33* exhibited no inhibition of growth on plates containing up to 50 mg/mL of SDS, beyond the aqueous solubility limit (data not shown). Also in accordance with prior literature, strain TY05 Δ*acrAB* harboring pBAD33* exhibited an MIC of less than 0.1 mg/mL. Complementation of TY05 Δ*acrAB* with pBAD33*-*acrAB* fully restored the MIC to greater than 50 mg/mL SDS (Figure S6). Complementation of TY05 Δ*acrAB* with pBAD33*-*emrAB*, pBAD33*-*mdtEF*, and pBAD33*-*mdtABCD* restored the MIC to less than 0.1 mg/mL, 0.1 mg/mL, and 0.1 mg/mL after one night incubation at 37°C, respectively (**Figure S7**). After two nights of incubation at 37°C, the MICs were 0.1 mg/mL, between 0.1 to 0.5 mg/mL, and 0.1 mg/mL, respectively. Functional expression was therefore validated in all four constructs.

While resistance can be conferred without overexpression of *tolC*, encoding the outer membrane component of each drug efflux pump, it is not known whether additional expression of *tolC* can improve observed MICs and function of inner membrane and periplasmic efflux pump components expressed on multicopy plasmids. Thus each drug efflux pump was also

cloned in an artificial operon with *tolC* harboring its native ribosome binding site. Interestingly, TY05 Δ*acrAB* harboring pBAD33*-*acrAB-tolC* completely lost the resistance to SDS observed with pBAD33*-*acrAB*, with an MIC of less than 0.1 mg/mL despite robust growth in LB containing chloramphenicol and L-arabinose (Figure S6). However, pBAD33*-*emrAB-tolC* and pBAD33*-*mdtABCD-tolC* conferred equivalent MICs of SDS as the non-*tolC* containing plasmids. Only pBAD33*-*mdtEF-tolC* exhibited an improved MIC over pBAD33*-*mdtEF* alone, with MIC increasing from between 0.1 to 0.5 mg/mL to 1.0 mg/mL after two nights incubation at 37°C (**Figure S7**). As a result, pBAD33*-*mdtEF-tolC* was the only *tolC* expressing construct selected to go forward with FFA plate MIC assays and overexpression in endogenous FFA overproducing strains.

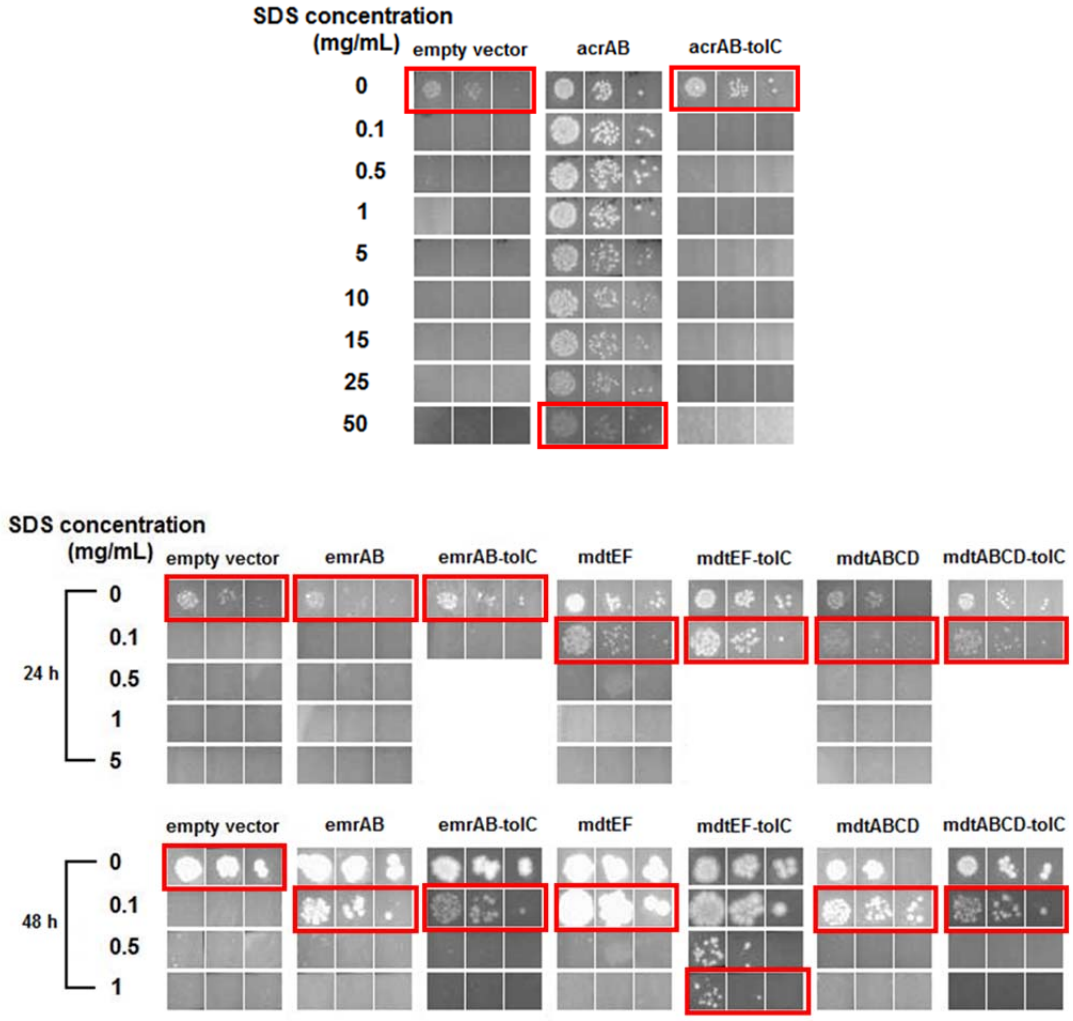

Figure S7: MIC assay for SDS against TY05 Δ*acrAB* expressing selected efflux pump system components on multicopy plasmids. TY05 Δ*acrAB* harbored empty vector as a negative control, or inner membrane/periplasmic linker components encoded in operons by themselves or in artificial operons with *tolC* expressed on a multicopy plasmid. (A) Expression of *acrAB* restores growth to TY05 Δ*acrAB* up to 50 mg/mL SDS, but expression of *acrAB-tolC* confers no resistance. (B) After 24 h, expression of *mdtEF*, *mdtEF-tolC*, *mdtABCD*, and *mdtABCD-tolC* confer resistance to 0.1 mg/mL SDS in TY05 Δ*acrAB*. After 48 h, growth is observed at 0.1 mg/mL for overexpression of *emrAB*, *emrAB-tolC*, *mdtEF*, *mdtABCD*, and *mdtABCD-tolC*, and up to 1 mg/mL for *mdtEF-tolC*.

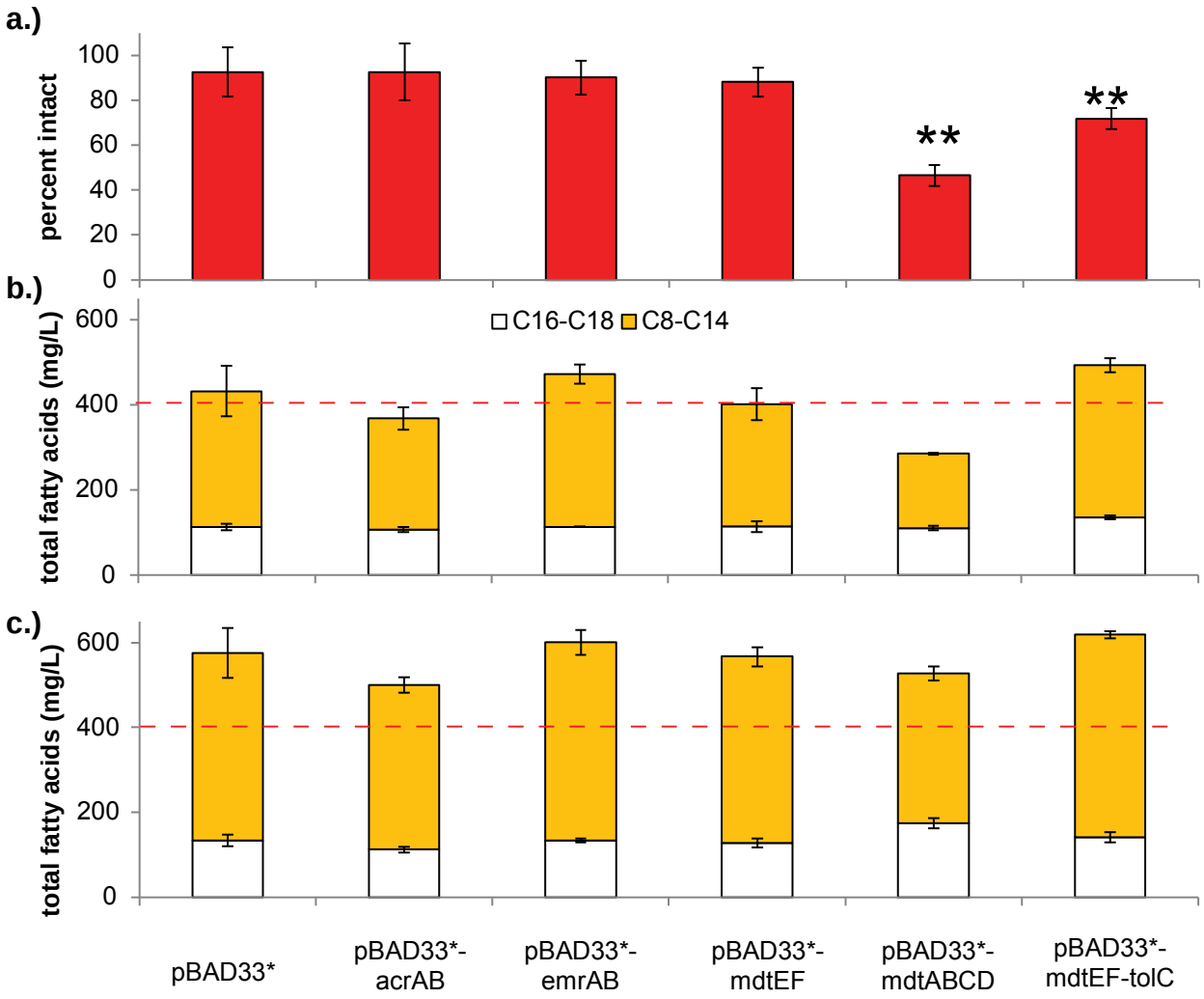

Figure S8: Analysis of *E. coli* TY05ara expressing selected efflux pumps on multicopy plasmids. Cultures of TY05ara harboring either empty vector pBAD33* or selected efflux pump genes cloned in pBAD33* were grown in LB + 0.4% glycerol + 34 µg/mL chloramphenicol + 0.5 mM each $MgSO_4$ and $CaCl_2$. (a) Percent intact cells determined by SYTOX Green staining. (b) C_8-C_{14} and C_{16}-C_{18} fatty acid titers 8 h post-inoculation. (c) Fatty acid titers 24 h post-inoculation. No improvements were observed over the negative control strain TY05ara/pBAD33*. Reduced C_8-C_{14} titers were evident from expression of MdtABCD from pBAD33* after 8 h.

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