Materials and Methods for Supplemental Data

Time course of *hilA* expression changes in response to external oleate. For the experiment addressing a time course of *hilA* regulation in response to oleate, bacteria were initially inoculated into LB with 1.5% Tergitol NP-40, grown overnight, then subcultured 1/100 in 25 ml HSLB with 1.5% Tergitol NP-40 in 50 ml flasks and grown for 2.5 h with aeration at 200 rpm. Then, (t=0) the resulting bacterial culture was aliquoted into 13 x 100 mm tubes (1.5 ml per tube) and grown in the absence or presence of 0.6 mM oleate on a roller drum for the indicated periods of time. Alternatively, bacteria were grown overnight in LB with 1.5% Tergitol NP-40 and 0.6 mM oleate, then subcultured 1/100 in 25 ml HSLB/1.5% Tergitol NP-40 and 0.6 mM oleate in 50 ml flasks and grown for 2.5 h with aeration at 200 rpm. Then, (t=0) the resulting bacterial culture was washed twice with the HSLB/1.5% Tergitol NP-40 medium, aliquoted into 13 x 100 mm tubes (1.5 ml per tube) and grown in the absence or presence of 0.6 mM oleate on a roller drum for the indicated periods of time, at which time the given sample was used to determine the β-galactosidase activity.

Analysis of LCFA content in culture supernatant: For the determination of LCFA content in the supernatants of the *fadL*, *fadD fadL*, and *fadD fadL tolC* mutants, bacteria were initially inoculated into MOPS EZ rich defined medium (Teknova Inc.), grown overnight, then subcultured 1/100 in 25 ml modified MOPS EZ medium (1x MOPS Rich Buffer, 1x ACGU solution, 0.04 mM K_2HPO_4, 1x Supplement EZ, 0.2% glucose) in 125 ml flasks and grown for 7 hours with aeration at 200 rpm. Triplicate cultures were grown for each mutant background.

Electrophoretic mobility shift assays (EMSA): EMSAs were performed using the SYBR Green EMSA kit (Thermo Fisher Inc.) according to manufacturer's instructions. Ten nM of a double stranded 210-bp DNA fragment of *hilC* promoter region (corresponding to –162 to +48) or 258-bp nonspecific DNA fragment were incubated for 20 min at RT with indicated amounts of purified HilD or HilC protein in a binding buffer (20 mM HEPES pH 7.3, 20 mM KCl, 1% glycerol, 1 mM DTT, 0.04 mM EDTA, 0.05% Tergitol NP-40) and 0.5x Novex Hi-Density TBE sample buffer. Oleate (or 90% ethanol at pH 6.8-7.0 as a control) was added to samples as indicated. The samples were separated on a 6% DNA retardation gel using 0.5x TBE running buffer according to the manufacturer's instructions (Invitrogen, Inc.).