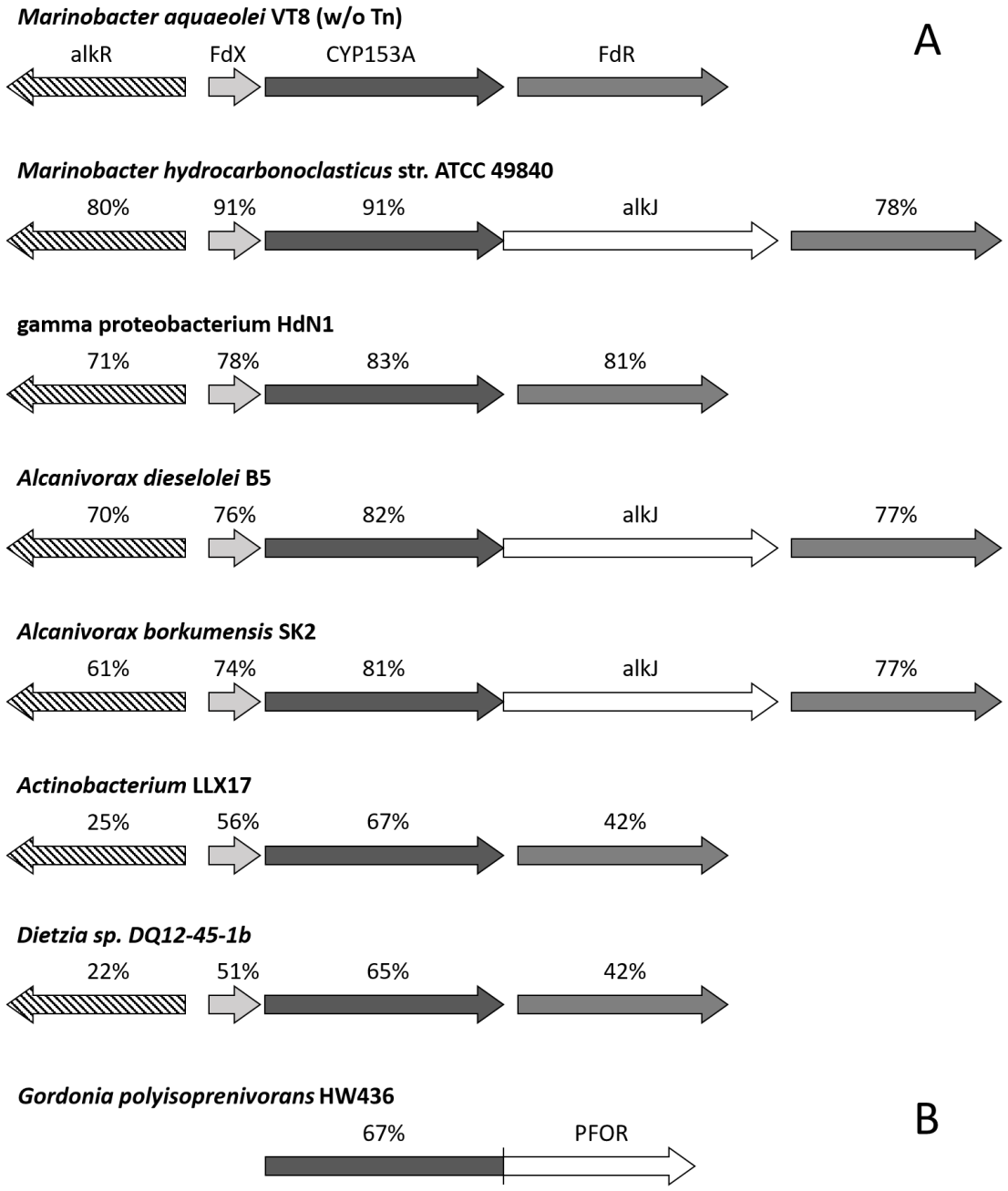

Figure S1 Protein sequence homologs of the CYP153A operon. (A) Sequence comparison of some CYP153 operons. CYP153 mostly include class I CYPs and comprise a cytochrome P450 enzyme, a ferredoxin and a ferredoxin reductase. The percentages indicate the protein sequence identities with homologs of *M. aquaeolei* VT8. CYP153 is the most conserved protein in the operon, while the regulator is the least conserved. Some operons contain a putative fatty alcohol dehydrogenase (alkJ). (B) CYP153 from *G. polyisoprenivorans* HW436 has a P450-PFOR fusion configuration. Percentages indicate protein sequence identities.

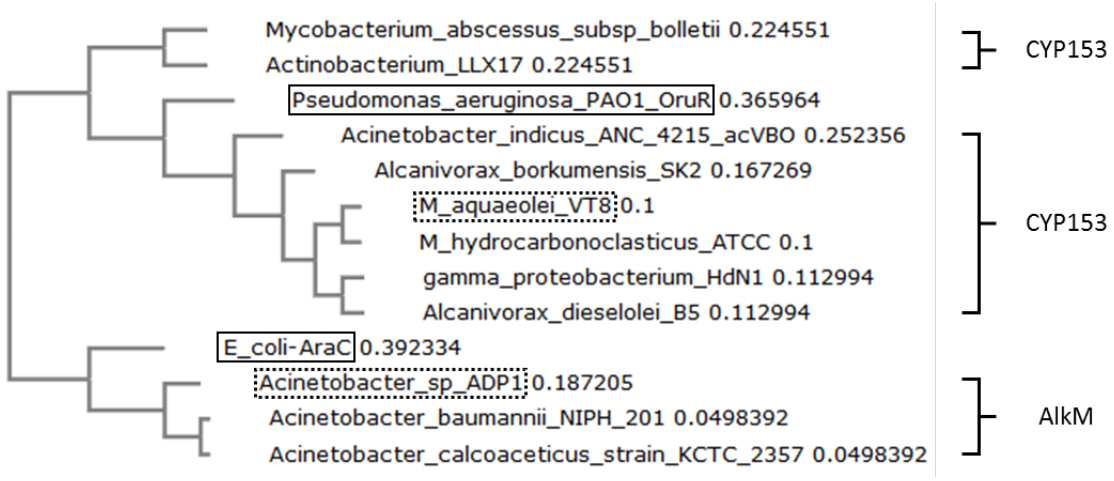

Figure S2 Phylogenetic tree analysis of the DNA sequences of alkane regulators. The sequences were analyzed by using online tool ClustalW2 with the UPGMA clustering method. The labels on the right side indicate the type of alkane-utilizing operons that have opposite orientations and are upstream of AraC-type regulators. The ornithine-utilizing regulator (OruR) of *P. aeruginosa* PAO1 and the arabinose regulator (AraC) of *E. coli*, marked with rectangles, were also used in this analysis. The characterized alkane response regulator (alkR) of *A. baylyi* ADP1 and the regulator of *M. aquaeolei* VT8 are bordered by dashed rectangles. The AraC/XylS regulators of alkane 1-monooxygenase (AlkM) and CYP153 cluster into different groups, which supports the idea that the AlkR in the CYP153A operon belongs to the AraC/XylS type regulators.

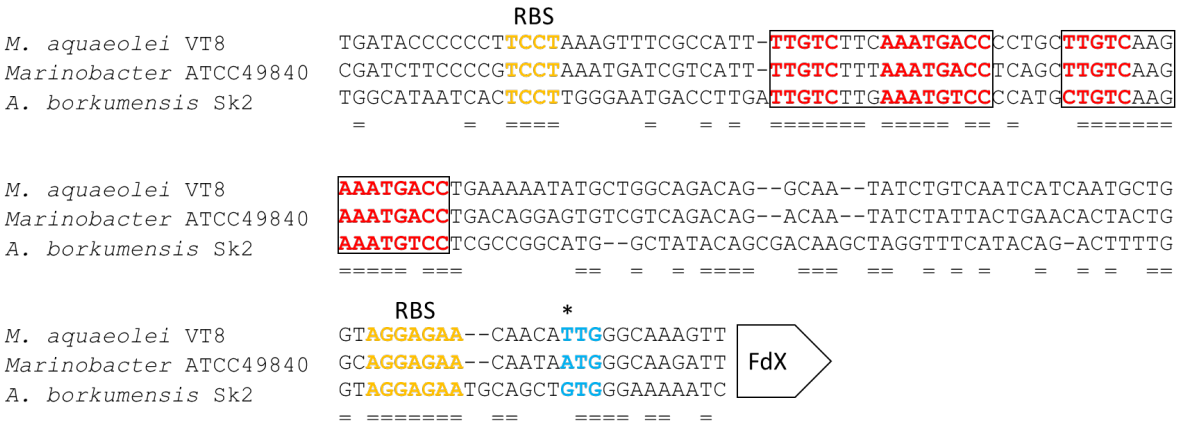

Figure S3 Multiple sequence alignment of putative CYP153A operon promoter regions. Comparing the intergenic sequences between the ferredoxin and the AraC-type regulator of the CYP153A system with their homologs from *M. hydrocarbonoclasticus* ATCC49840 (*cyp153a* 93% identity) and *Alcanivorax borkumensis* SK2 (*cyp153a* 83% identity) by using ClustalW. The direct repeat (TTGTCNNNAAATGACC, marked by rectangles) is relatively conserved. The start codon of ferredoxin is marked by an asterisk. Alternative start codons (TTG, GTG) are common among ferredoxins in CYP153A operons.

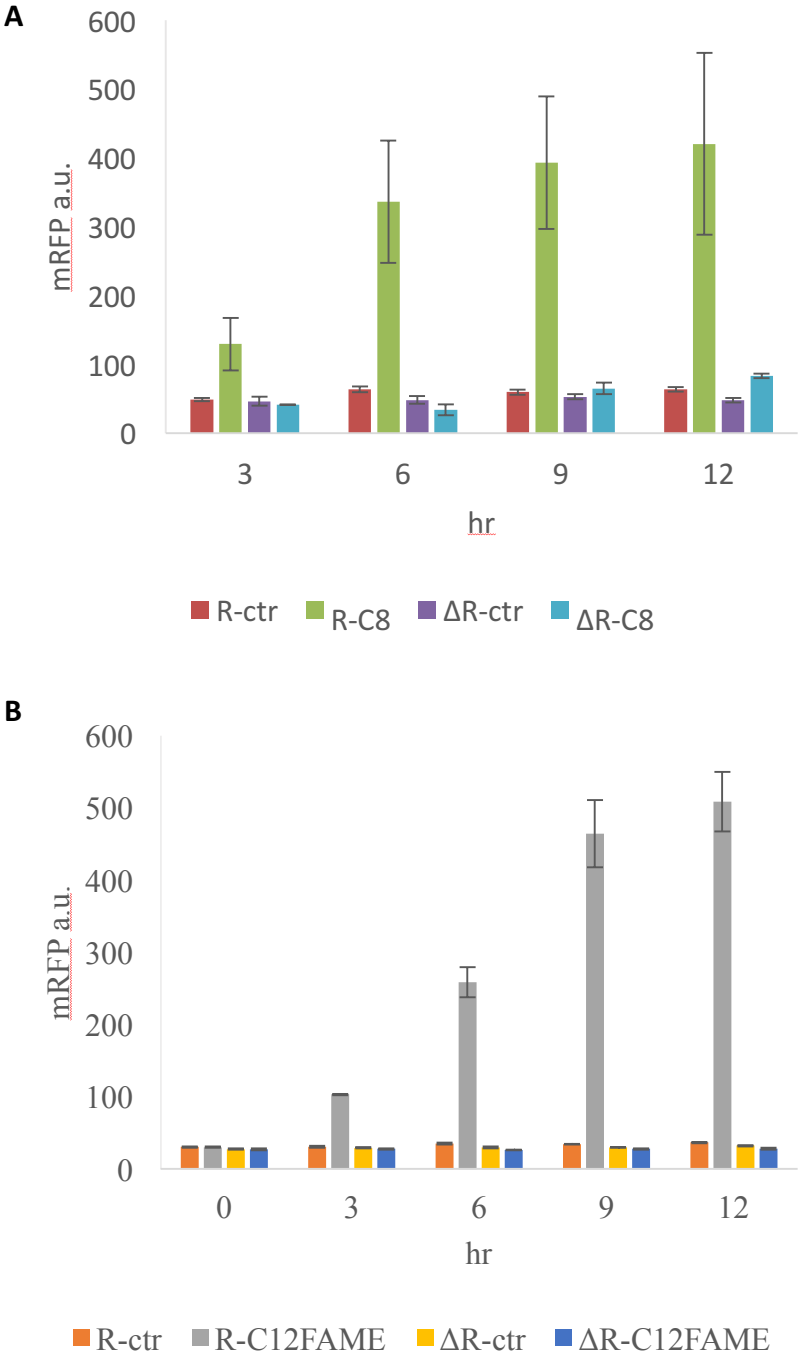

Figure S4. Partial deletion disables the AlkR induction system. The putative AraC/XylS-type transcription regulator is responsible for induction by (A) octane and (B) C12-FMAE. It may act as a similar activator as other AraC/XylS type proteins. R/ΔR: *E. coli* carried mRFP reporter plasmid with AlkR or without AlkR. –ctr/-c8/-C12FMAE: E. coli induced by water control, octane, or dodecyl methyl ether.

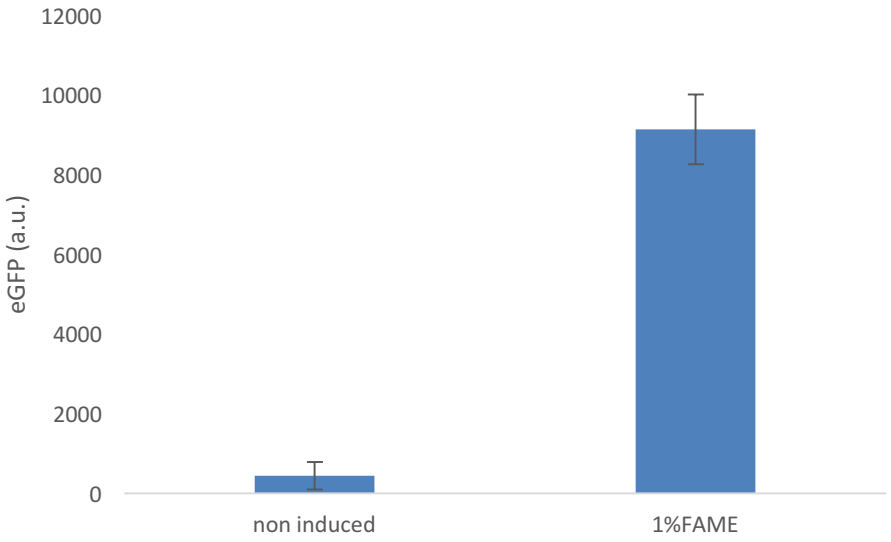

Figure S5 eGFP signals 6 hours after induction by 1% FAME (octyl methyl ether). Translational fusion of eGFP with the putative N-terminus of the interrupted ferredoxin showed that the operon contained a functional RBS and start codon.

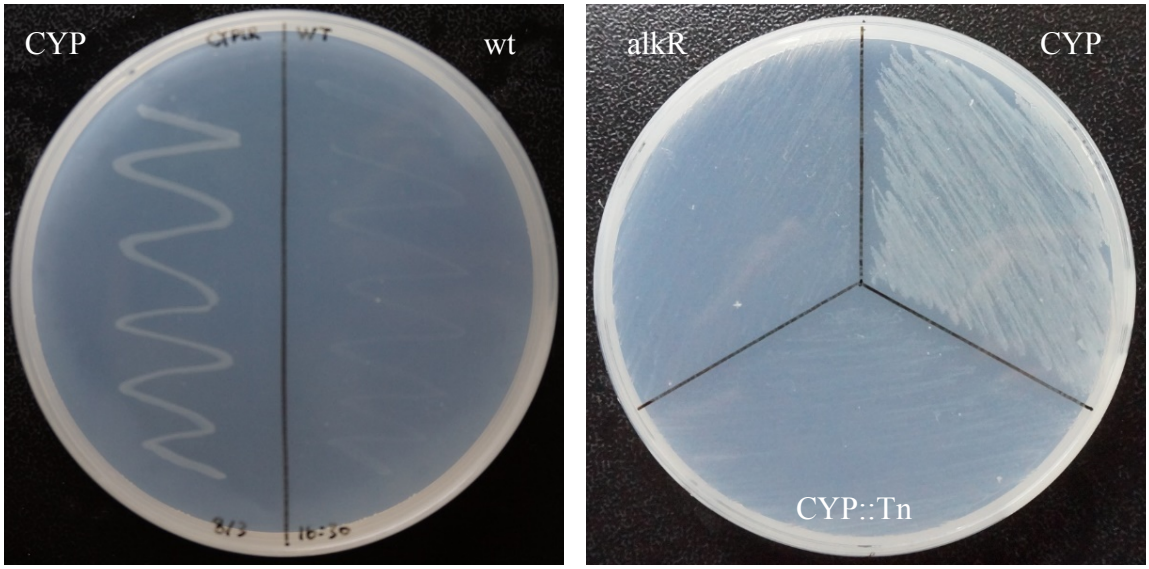

Figure S6 Octane growth assay. Reconstituted CYP153A system enables *P. putida* mt-2 to use octane vapor as a sole carbon source. These pictures were taken 4 days after incubation began. CYP, *P. putida* mt-2 with restored operon; wt, wild-type *P. putida* mt-2; alkR, regulator-only vector; CYP::Tn, the operon with inserted transposon, which originally existed in *M. aquaeolei* VT8.

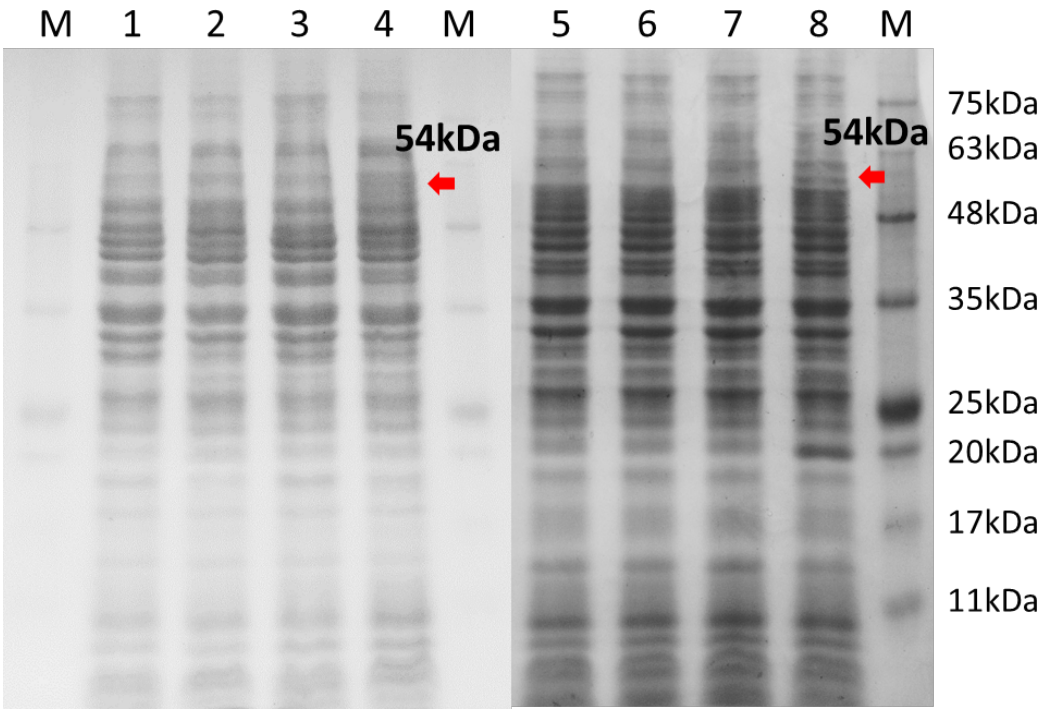

Figure S7 CYP153A expressed upon induction in *E. coli*. Lanes 1-2: *E. coli* carrying rhamnose-inducible plasmid without the operon; lanes 3-4: *E. coli* carrying rhamnose-inducible CYP153A operon; lanes 5-6: *E. coli* carrying alkane-inducible vector without the operon; lanes 7-8: *E. coli* carrying entire restored CYP153A operon. Lanes 1, 3, 5 and 7: cells without any induction as control experiments. Lanes 2 and 4: cells were induced with 0.2% (w/v) rhamnose; lanes 6 and 8: cells were induced by 1% (v/v) methyl laurate. The 54 kDa band indicates CYP153A protein expression.

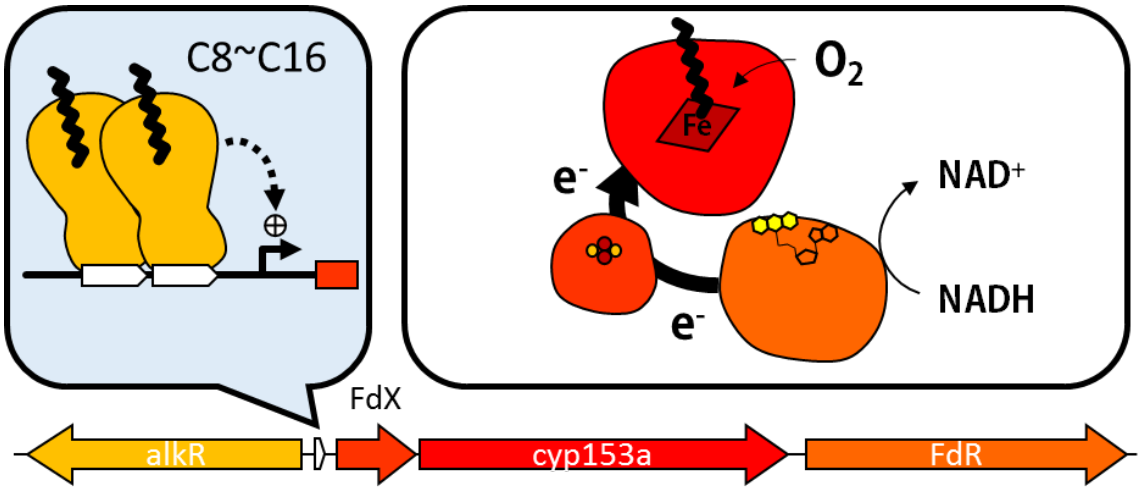

Figure S8 Scheme of our reconstituted functional CYP153A system. AlkR, AraC-type transcriptional regulator maqu_0596; FdX, ferredoxin; *cyp153a*, cytochrome P450 CYP153A; and FdR, ferredoxin reductase.

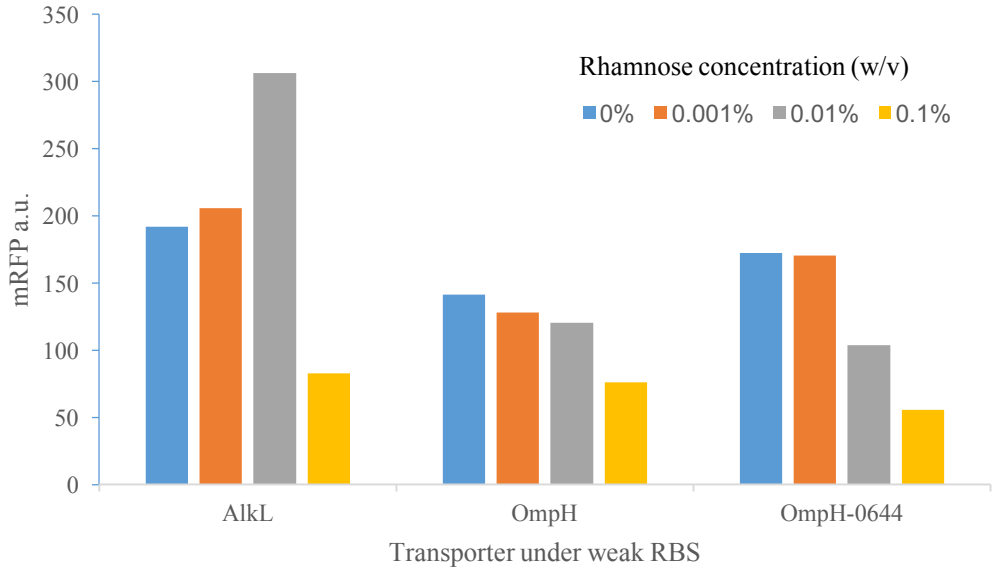

Figure S9 Differences in enhancement while expressing AlkL, OmpH, and OmpH-0644 under a weak RBS in *E. coli* carrying the reporter plasmid.