Supplemental Information

Experimental Procedures:

Measurement of bacterial growth curve
Bacterial cells were initially cultured in LB broth at 37°C for 16 hours. At the next day, the overnight cultures were adjusted to O.D.\textsubscript{600}=1.2 and inoculated into 5 mL fresh LB broth with 1:500 dilution. The O.D.\textsubscript{600} values for the bacterial growth curve were automatically measured every 30 min using an OD-Monitor C&T (Taitec, Saitama, Japan) as described (1).

Construction of EHEC deletion mutants
The isogenic gene deletion mutants were constructed by the Lambda Red-mediated recombination system as described (2, 3). The Lambda Red recombinase expression plasmid pKD46 is a temperature-sensitive plasmid, and the lambda red proteins were induced with 10 mM L-arabinose. This method was performed using primers containing the sequence for 30 bp homology to the target gene and 20 bp to amplify a kanamycin or chloramphenicol resistance gene from pKD4 or pKD3. The primers used in the mutant construction are listed in Table S2. The antibiotic resistance genes were flanked by recombinase FLP recognition target (FRT) sites, and directly repeated FRT sites were used for antibiotic resistance gene removal with pCP20. For generation of the EHEC strain EDL933 deletion mutants, the purified DNA fragments were electroporated into EDL933 harboring pKD46 cells. After electroporation, cells were incubated with 2XYT at 37°C for 1 hour, and plated on an LB agar plate containing kanamycin or chloramphenicol. The plates were incubated at 37°C for antibiotic screening and to induce the loss of pKD46. In order to generate EDL933 multiple gene mutants or prevent the polar effects on upstream and downstream gene expression of target genes, it is necessary to remove the resistance cassette with pCP20. The Flp recombinase expression plasmid pCP20 is also a temperature-sensitive plasmid, and the expression of Flp recombinase is induced at 43°C (2-4). Flp recombinase recognizes the FRT sites and removes the FRT site-flanked antibiotic resistance gene, generating an in-frame deletion mutant. The selected colonies were sensitive to Ampicillin and Kanamycin or chloramphenicol for absence of pCP20 and the resistance gene.
Figure S1. Screening of the EDL933 transpososome mutant library.

*C. elegans* *glp-4 (bn2)* L1 stage larvae were cultured on the Enriched Nematode Growth (ENG) medium plates at the restrictive temperature (25°C) at Day 1. At the same day, the EDL933 transpososome mutant library, stored in 96-well plates and in -80°C freezers, was replicated in LB broth containing 50 µg/mL Kanamycin (Kan) and put in a 37°C incubator for 16 to 18 hours. At Day 2, the entire library was triplicated in 96-well plates containing LB broth with 50 µg/mL Kan and cultured at 37 °C for another 16 to 18 hours. At Day 3, when *C. elegans* *glp-4 (bn2)* animals reached to L4 larvae/young adult stage, the worms were washed off from ENG plates by M9 buffer and collected. These worms were mixed with each transposon mutant clones in 96-well plated, which was centrifuged and resuspended in S medium. Each well contained approximately 20 worms. Then, the 96-well plates were placed at 25°C with shaking at 70 rpm. After 8 days, the O.D.595 values of each well were measured. The O.D.595 value was close to 0.5 when worms were cultured with *E. coli* strain OP50 (as negative control). In contrast, the O.D.595 value was around 1.0 when the worms were fed with EHEC wild-type EDL933 (as positive control). The hits/candidates with a decreased pathogenic phenotype toward *C. elegans* were selected with the O.D. value that was significantly lower compared to the EHEC wild-type EDL933 positive controls (*P*<0.05).
Figure S2. Growth curves of the EHEC strains.
The growth curves of the wild-type EHEC strain EDL933 (EDL933), the isogenic *sdhA* transposon mutant [YQ413 (*sdhA::Tn5*)], and the isogenic *sdhA* deletion mutant (EDL933:*ΔsdhA*) were measured.
Anaerobic metabolism is dispensable for the full virulence of EHEC in *C. elegans*

During anaerobic metabolism, the TCA cycle is repressed and nitrate catalyzed by nitrate reductase (Nar) and fumarate catalyzed by fumarate reductase (Frd) can both act as the alternative terminal electron acceptors other than oxygen (5); or alcohol dehydrogenase, encoded by the *adhE* gene, can regenerate NAD⁺ for glycolysis and control fermentation in *E. coli* (6). Moreover, the transcriptional regulator Fnr (fumarate/nitrate reduction regulator) is required for anaerobic respiration and controls the switch from aerobic to anaerobic respiration (7), and the ribonucleotide reductase class III, encoded by *nrdD* and *nrdG*, is essential for a strictly anaerobic environment in *E. coli* (8). To test whether anaerobic metabolism, including anaerobic respiration and fermentation, also plays roles in the pathogenesis of EHEC in *C. elegans*, five isogenic mutants with *narHJI*, *frdA*, *adhE*, *fnr*, and *nrdDG* deletion (EDL933:ΔnarHJI, EDL933:ΔfrdA, EDL933:ΔadhE, EDL933:Δfnr, and EDL933:ΔnrdDG) were generated and tested. We noted that these isogenic mutants were as toxic as the parental wild-type EDL933 (Figure S3). Given the potential redundancy of these genes in controlling anaerobic metabolism, a compound mutant was also generated. Our results showed that the isogenic EDL933:ΔnarHJIΔfrdAΔadhEΔfnrΔnrdDG mutant strain was as toxic as the wild-type EDL933 (Figure S3). Together, our current data suggested that anaerobic metabolism is dispensable for the full virulence of EHEC in *C. elegans*.

![Figure S3. Deletion of genes involved in anaerobic metabolism did not alter EHEC toxicity in *C. elegans*.](image-url)
The survival of N2 worms fed with the wild-type EDL933 (EDL933) and the isogenic deletion strains of *narHJI* (EDL933:*ΔnarHJI*), *frdA* (EDL933:*ΔfrdA*), *adhE* (EDL933:*ΔadhE*), *fnr* (EDL933:*Δfnr*), and *nrdDG* (EDL933:*ΔnrdDG*) were examined. Deletion of *narHJI* (median N2 lifespan = 6.0 ± 0.1 days, *P*=0.205), *frdA* (median N2 lifespan = 6.7 ± 0.6 days, *P*=0.129), *adhE* (median N2 lifespan = 6.0 ± 0.1 days, *P*=0.413), *fnr* (median N2 lifespan = 6.0 ± 0.1 days, *P*=0.448), and *nrdDG* (median N2 lifespan = 6.5 ± 0.7 days, *P*=0.908) were as toxic as the parental wild-type EDL933 (median N2 lifespan = 6.2 ± 0.5 days). "ns" represents no statistically significant difference examined by the Log-rank test.
The effect of fumarate is specific to EHEC

The survival curves of *C. elegans* animals did not change when fed on the succinate or fumarate treated OP50 (Figure S4A). These results suggested that the effect of fumarate was on EDL933:ΔsdhA mutant directly. We also generated the isogeneic *sdhA* mutant strain of *E. coli* OP50 (OP50:ΔsdhA) to examine whether the effect of fumarate is specific to EHEC. Our results showed that the survival curves of *C. elegans* animals fed on the wild-type OP50 and the OP50:ΔsdhA mutant were similar (Figure S4B). Moreover, the survival curves of *C. elegans* animals fed on succinate or fumarate treated OP50:ΔsdhA were similar to the untreated control, which suggested that the *sdhA* gene is specifically required for the pathogenesis of EHEC in *C. elegans*.

Figure S4. Supplement of *E. coli* OP50 and OP50:ΔsdhA with succinate or fumarate did not alter *C. elegans* lifespan.

(A) The survival curves of worms fed with the wild-type OP50 strain cultured with 2.5 mM succinate (OP50+Succinate) or fumarate (OP50+Fumarate) were examined. Animals on OP50 treated with succinate (OP50+Succinate, N2 median lifespan = 18.5 ± 1.5 days, *P*=0.72) or fumarate (OP50+Fumarate, N2 median lifespan = 17.8 ± 0.49 days, *P*=0.40) shown a similar lifespan compared to that on OP50 (OP50, N2 median lifespan = 18.67 ± 0.42 days). (B) The survival curves of worms fed with the wild-type OP50 strain, and OP50 with isogenic deletion strain of *sdhA* (OP50:ΔsdhA) cultured with 2.5 mM succinate (OP50:ΔsdhA+Succinate) or fumarate (OP50:ΔsdhA+Fumarate) were examined. Worms on the OP50:ΔsdhA strain (OP50:ΔsdhA, N2 median lifespan = 20.0 ± 1.4 days, *P*=0.627) exhibited similar lifespan compared to the wild-type OP50.
strain (OP50, N2 median lifespan = 20.5 ± 0.7 days) toward C. elegans animals. Worms on succinate-treated OP50:ΔsdhA strain (OP50:ΔsdhA+Succinate, N2 median lifespan = 20.0 ± 0.1 days, P=0.842) and fumarate-treated OP50:ΔsdhA strain (OP50:ΔsdhA+Fumarate, N2 median lifespan =20.5 ± 0.7 days, P=0.878) all exhibited similar lifespan compared to the untreated control (OP50:ΔsdhA, N2 median lifespan = 20.0 ± 1.4 days). "ns" represents no statistically significant difference examined by the Log-rank test.
The three putative C4-dicarboxylates sensor-regulator systems are dispensable

The *dcuSR* operon (also known as *yjdHG*) encodes a two-component sensor-regulator system (DcuS-DcuR) which can sense fumarate and lead to activation of the fumarate-succinate antiporter DcuB expression in *E. coli* (9, 10). If fumarate restores *sdhA* mutant toxicity/virulence through the DcuSR two-component system, deletion of *dcuSR* in the *sdhA* mutant background cannot restore its toxicity after supplement of fumarate. We therefore generated the *sdhAdcuSR* isogenic mutant and examined its toxicity to *C. elegans* under fumarate supplement. As shown in Figure S5A, the toxicity of *sdhAdcuSR* mutant to *C. elegans* was significantly attenuated compared with wild-type EHEC (*P*<0.0001) but was similar to the *sdhA* single mutant (*P*=0.151). Moreover, addition of 2.5 mM fumarate not only restored the toxicity of *sdhA* mutant but also the *sdhAdcuSR* mutant which suggested that the *dcuSR* two-component system is not involved in sensing fumarate to regulate the virulence of EHEC.

Another DctS-DctR two-component system, which encoded by *dctS* and *dctR* genes, is required for high-affinity C4-dicarboxylate transport in *Rhodobacter capsulatus* (9, 11). We blasted the amino acid sequence of DctS and DctR to the EDL933 amino acid sequence and identified YhiF (Z4909, *yhiF*) as a close homolog of DctR, but could not identify any homolog of DctS. The DctB-DctD sensor-regulator controls the expression of the *dctA* gene encoding C4-dicarboxylate transporter DctA in *Rhizobia* (11). We also blasted the amino acid sequence of DctB and DctD to EDL933 protein sequence and identified HyfR (Z3751, *hyfR*) as having the closest homology to DctD. However, we could not identify any DctB homolog in EDL933. Therefore, we generated the isogenic mutant of *dctR* (*yhiF*) and *dctD* (*hyfR*) in the *sdhA* mutant background to examine whether fumarate regulates EDL933 virulence through SdhA via these two-component systems. As shown in Figure S5B, *dctRsdhA* double mutant is less toxic to *C. elegans* compared with wild-type EHEC (*P* < 0.0001) but is similar to the *sdhA* single mutant (*P*=0.96). Supplement of 2.5 mM fumarate to the *dctRsdhA* double mutant restored its toxicity to that of the *sdhA* single mutant (*P*=0.57), suggesting that the DctS-DctR two-component sensing pathway is not required for fumarate to regulate EHEC toxicity.

We also generated *dctD* isogenic mutant in the *sdhA* mutant background and examined its toxicity toward *C. elegans* when supplied with 2.5 mM fumarate. In the same manner as the *dctRsdhA* double mutant, addition of fumarate to the *dctDsdhA* double mutant rescued its toxicity to that of the *sdhA* single mutant (*P*=0.86) (Figure S5C).
Figure S5. Deletion of the putative two-component systems in C4 dicarboxylates regulation did not affect the capability of fumarate to restore the toxicity of the EHEC sdhA mutant.

(A) The survival of N2 worms fed with the wild-type strain (EDL933) and the isogenic deletion strains of sdhA (EDL933:ΔsdhA), the sdhA and dcuSR triple mutant (EDL933:ΔsdhAΔdcuSR) and mutants treated with 2.5mM fumarate, respectively (EDL933:ΔsdhA+Fumarate and EDL933:ΔsdhAΔdcuSR+Fumarate), were examined. The virulence of sdhA and dcuSR triple mutant treat with 2.5mM fumarate (EDL933:ΔsdhAΔdcuSR+Fumarate, median N2 lifespan = 9 days) was similar to sdhA mutant treated with 2.5 mM fumarate (EDL933:ΔsdhA+Fumarate, median N2 lifespan = 8 days, P=0.52). (B) The survival of N2 worms fed with the wild-type strain (EDL933)
and the isogenic deletion strains of $sdhA$ (EDL933:$\Delta sdhA$), the $sdhA$ and $dctR$ double mutant (EDL933:$\Delta sdhA\Delta dctR$) and mutants treated with 2.5mM fumarate, respectively (EDL933:$\Delta sdhA$+Fumarate and EDL933:$\Delta sdhA\Delta dctR$+Fumarate) were examined. The virulence of $sdhA$ and $dctR$ double mutant treated with 2.5 mM fumarate (EDL933:$\Delta sdhA\Delta dctR$+Fumarate, median N2 lifespan = 7.3 ± 0.6 days) was similar to $sdhA$ mutant treated with 2.5mM fumarate (EDL933:$\Delta sdhA$+Fumarate, median N2 lifespan = 7.4 ± 0.5 days, $P=0.57$). (C) The survival of N2 worms fed with the wild-type strain (EDL933) and the isogenic deletion strains of $sdhA$ (EDL933:$\Delta sdhA$), the $sdhA$ and $dctD$ double mutant (EDL933:$\Delta sdhA\Delta dctD$) and mutants treated with 2.5 mM fumarate, respectively (EDL933:$\Delta sdhA$+Fumarate and EDL933:$\Delta sdhA\Delta dctD$+Fumarate), were examined. The virulence of $sdhA$ and $dctD$ double mutant treat with 2.5 mM fumarate (EDL933:$\Delta sdhA\Delta dctD$+Fumarate, median N2 lifespan = 8 days) was similar to $sdhA$ mutant treated with 2.5 mM fumarate (EDL933:$\Delta sdhA$+Fumarate, median N2 lifespan = 8 days, $P=0.86$). “ns” represents no statistically significant difference examined by the Log-rank test.
**Tables**

**Table S1. Nematode strains used in this study.**

| Strain  | Relevant characteristics                                                                 | Source or reference |
|---------|------------------------------------------------------------------------------------------|---------------------|
| N2      | *C. elegans* wild-type strain                                                             | (12)                |
| GK454   | *unc-119(ed3), dkl1s247[Pact-5::mCherry::HA::act-5, unc119(+)]; mCherry::ACT-5 expression* | (13)                |
Table S2. Bacterial strains used in this study.

| Strain             | Description                                                                 | Source or reference |
|--------------------|-----------------------------------------------------------------------------|---------------------|
| OP50               | uracil auxotroph and laboratory food source for *C. elegans*               | (12)                |
| EDL933             | *E. coli* O157:H7 isolated from raw hamburger meat                         | (14)                |
| HER1266            | *E. coli* O157:H7 isolated from human stool                                | (15)                |
| YQ413              | *sdhA*::Tn5, Tn5 transposon mutant inserted in the *sdhA* gene of EDL933    | this study          |
| EDL933:ΔsdhA       | EDL933 isogenic mutant with *sdhA* gene deleted; Kan^R^ kick out            | this study          |
| EDL933:ΔsdhC       | EDL933 isogenic mutant with *sdhC* gene deleted; Kan^R^                     | this study          |
| EDL933:ΔsdhD       | EDL933 isogenic mutant with *sdhD* gene deleted; Kan^R^                     | this study          |
| EDL933:ΔsdhB       | EDL933 isogenic mutant with *sdhB* gene deleted; Kan^R^                     | this study          |
| EDL933:ΔsdhCΔsdhD  | EDL933 isogenic mutant with *sdhCDAB* operon deleted; Kan^R^                | this study          |
| EDL933-pQE30       | EDL933 transformed with pQE30; Amp^R^                                       | (16)                |
| EDL933:ΔsdhA-pQE30 | EDL933 isogenic mutant with *sdhA* gene deleted; Kan^R^ kick out, and transformed with pQE30; Amp^R^ | this study          |
| EDL933:ΔsdhA-pWF134| EDL933 isogenic mutant with *sdhA* gene deleted; Kan^R^ kick out, and transformed with pWF134; Amp^R^ | this study          |
| EDL933:ΔsdhA-pWF134| EDL933 isogenic mutant with *sdhA* gene deleted; Kan^R^, and complement with *sdhCDAB* by transformation with pWF134; Amp^R^ | this study          |
| EDL933:ΔsdhCDAB-pWF134 | EDL933 isogenic mutant with *sdhCDAB* gene deleted; Kan^R^, and complement with *sdhCDAB* by transformation with pWF134; Amp^R^ | this study          |
| EDL933 *sdhA*::Tn5-pWF134 | EDL933 transposon inserted in *sdhA* gene; Kan^R^, and complemented with *sdhCDAB* by transformation with pWF134; Amp^R^ | this study          |
| OP50:ΔsdhA         | OP50 isogenic mutant with *sdhA* gene deleted; Kan^R^                       | this study          |
| Strain | Description | Source or reference |
|--------|-------------|---------------------|
| EDL933:ΔicdA | EDL933 isogenic mutant with icdA gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔsucAΔsucB | EDL933 isogenic mutant with sucA gene and sucB gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔsucCΔsucD | EDL933 isogenic mutant with sucC gene and sucD gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔfrdA | EDL933 isogenic mutant with frdA gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔfumCΔfumA | EDL933 isogenic mutant with fumC gene and fumA gene deleted; Kan<sup>R</sup> | this study |
| EDL933:Δmdh | EDL933 isogenic mutant with mdh gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔgltA | EDL933 isogenic mutant with gltA gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔygfH | EDL933 isogenic mutant with ygfH gene deleted; Cm<sup>R</sup> | this study |
| EDL933:ΔsdhAΔygfH | EDL933 isogenic mutant with sdhA gene and ygfH gene deleted; Kan<sup>R</sup> Cm<sup>R</sup> kick out | this study |
| EDL933:ΔarcA | EDL933 isogenic mutant with arcA gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔarcB | EDL933 isogenic mutant with arcB gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔarcAΔarcB | EDL933 isogenic mutant with arcA gene and arcB gene deleted; Kan<sup>R</sup> Cm<sup>R</sup> | this study |
| EDL933:Δfmr | EDL933 isogenic mutant with fmr gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔnarHΔnarJ ΔnarI Δfmr ΔadhE | EDL933 isogenic mutant with narH gene, narJ gene, and narI gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔadhE | EDL933 isogenic mutant with adhE gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔnrdDΔnrdG | EDL933 isogenic mutant with nrdD gene and nrdG gene deleted; Kan<sup>R</sup> | this study |
| EDL933:ΔnarHΔnarJ ΔnarIΔfmrΔadhE | EDL933 isogenic mutant with narHnarJnarI gene deleted; Kan<sup>R</sup> kick out, fmr gene; Kan<sup>R</sup>, and adhE gene; Cm<sup>R</sup> | this study |
| EDL933:ΔsdhAΔtnaA | EDL933 isogenic mutant with sdhA gene; Kan<sup>R</sup>, and tnaA gene deleted; Kan<sup>R</sup> | this study |
**Table S3. Plasmids used in this study.**

| Plasmid     | Relevant characteristics                                           | Source or reference |
|-------------|-------------------------------------------------------------------|---------------------|
| pFPV25.1    | Vector for constitutive GFP expression; *rpsM::gfpmut*; Amp<sup>R</sup> | (17)                |
| pKD46       | Red recombinase expression; Amp<sup>R</sup>                       | (2, 18)             |
| pKD3        | Template plasmid for Cm<sup>R</sup> cassette                     | (2)                 |
| pKD4        | Template plasmid for Kan<sup>R</sup> cassette                    | (2)                 |
| pQE30       | Amp<sup>R</sup>, T5 expression vector                             | Qiagen, USA         |
| pCP20       | FLP recombinase expression; Amp<sup>R</sup> Cm<sup>R</sup>         | (2)                 |
| pWF134      | *sdhCDAB* expressing plasmid; Amp<sup>R</sup>                     | this study          |
Table S4. Primers used in cloning, mutant construction, and qRT–PCR in this study.

| Name            | Oligonucleotides                                      |
|-----------------|-------------------------------------------------------|
| **Primers used for cloning (5’ to 3’)**                  |                                                       |
| pQE30-sdhCDAB F5| ACATGCATGCTTAAGGTCTCCTCCTTAGCGGCTGAGCTGCTTC          |
| pQE30-sdhCDAB R3| ACGCGTCGACGCGCATCCGGCACTGGTTG                       |
| sdhA F5         | GGATTCGTTGTGGTGGGAGTGAGGTAGGAGTGGGCTGGAGCTGCTTC      |
| sdhA R3         | CATTTTCTGTTCTCCGCAATTAGTACGCATTAGATATCCTCCTTAG       |
| sdhA Up R3      | TCATCACACCGCCACACACTGCAATCC                         |
| Check sdhA F5   | CTATCTGGAAGAAACATTCG                                |
| Check sdhA R3   | AGGGTGTAATCCTGACATAC                                 |
| sdhB F5         | GTACTTACTAATGCGGAGACAGAAATGTAGGGCTGGAGCTGCTTC      |
| sdhB R3         | TCTTATCAGGGCTACGGTATTACGCATTAGCATGATATCCTCCTTAG     |
| sdhB Up R3      | TCATGAATAAGCGCCACACTGAGTAC                          |
| Check sdhB F5   | AGCATATACTTCCTTGGCTTC                               |
| Check sdhB R3   | ATACTACACGCAAGTATG                                  |
| sdhC F5         | ATAAAGAACAGCATGTTGGCCGTATTCATGAGGTAGGGCTGGAGCTGCTTC |
| sdhC R3         | CTAATGCACGGAGGCGTTAGTACCAGCAGCATGATATCCTCCTTAG     |
| sdhC Up R3      | TCATGAATAAGCGCCACACTGATGAC                         |
| Check sdhC F5   | CTAATAACTGTCCCCAGATAA                              |
| Check sdhC R3   | ATAAATACGCAAGTACAGT                                 |
| sdhD F5         | CTTTCACCTTCTCCGAGGGAGTCTCGATAGGGCAGCTGGAGCTGCTTC   |
| sdhD R3         | CTCTGACTGGCAATTTCATCCACCCACCATATGCAATATCCTCCTTAG   |
| sdhD Up R3      | CCATACGAGGACTCTCTCGAGAGGAATGGAAAA                  |
| Check sdhD F5   | TATCACGTGCTCGTAGGTAT                                |
| Check sdhD R3   | CCGGTTTTTACACATATATAC A                              |
| acnB F5         | GAATACCGTAAGCAGTACGTCAGGTAGGGCTGGAGCTGAGCTGCTTC    |
| acnB R3         | AGTCTGGGAAATTCAGCCAATCTTGCTTTCTC CATAT GAATA TCTC CT TAG |
| acnB Up R3      | GCCACGTCAGTACGTAGCTCCTACGGTAGAT                     |
| Check acnB F5   | TATCAGTAATCTCGATCAGTGAGTATCAAG                      |
| Check acnB R3   | TTCGTCGTAAGTAGTCATCC                                |
| icdA F5         | AAAGTAGTTGTTCTCGGCAACAGAAGGTTAGGGCTGGAGCTGCTTC     |
| icdA R3         | CTTGTATGAGTACGGTACCAAAACTCTGAGACACATATGAAATATCCTCCTT AG |
| icdA Up R3      | CTTTCTGCTTTGTCGGGAAACACTACTTT                        |
| Check icdA F5   | TATGTTACGCTACAGTAAC                                  |
| Check icdA R3   | CATTACCGTCACACTCC                                  |
| Gene | Region | Primer 1 | Primer 2 |
|------|--------|----------|----------|
| sucA | F5     | AGCGCTTTGAAAGCCTGGTTGAGTGTGGCTGGAGCTGCTTC |  |
| sucB | R3     | CAGCAGACAGACGCTGGATCCTCCAGCAACATATGAGATATCCTCCTTAG |  |
| sucA | Up R3  | AGGAAGAGTCTCAACCAGGCTTTTCAAAGCGCT |  |
| Check| sucA F5| AGGTATCAGCTGCTATAG |  |
| Check| sucB R3| ACGTGAACATCGGCCTACAA |  |
| sucC | F5     | CGATTACTGAAGGATGGACAGACACATGAGTGGCTGGAGCTGCTTC |  |
| sucC | R3     | TATCAATTAAATGGACACATTATTTCCCCCTCCCATAGAATATATCCTCCTTAG |  |
| sucC | Down F5| GAGGGGAATAATGTCACCTTTTAAAGTATA |  |
| Check| sucC F5| TGGTAAACGATCAAGATGGT |  |
| Check| sucC R3| GGTGATAATCAATTTTGATG |  |
| sucD | F5     | GGGTTTGCGCCAGCGAGGGGAAATAATGTTGCTGGAGCGGTGCTTCT |  |
| sucD | R3     | ATTTCTTATTACAGATATTTATTGACAACCATATGAGATATCCTCCTTAG |  |
| sucD | Down F5| GGGTGAAATAATGTCATATAG |  |
| Check| sucD F5| GACGACGATCCCTCCAGATGGT |  |
| Check| sucD R3| ATCCCTCTAAGAATTTTTGC |  |
| frdA | F5     | CAAACAGACTGAGATGGCAGGAGGAGTACTTACATGAGTGGCTGGAGCTGCTTC |  |
| frdA | R3     | ATTTTCAAGTTTTTCTACACATGGCGGTCATATGAGATATCCTCCTTAG |  |
| frdA | Up R3  | GCCGGAACGTTATGCTGAGATG |  |
| Check| frdA F5| TCTCGTCAATTTTCAGACTT |  |
| Check| frdA R3| GGGTCTGGGCTATTTTATCC |  |
| fumC | F5     | AATTAATCGTGGAGAGTAGAAGGAGGACCTTAAATGGGTGTAGGCTGGAGCTGCTTC |  |
| fumC | R3     | GCCACCTGTATGTGAGTCAAGATGAGTGGCTGGAGCTGCTTC |  |
| fumC | Down F5| GCCGGGCGTTAATCTGCAACATACAGGTGC |  |
| Check| fumC F5| TTTTACATGGCAGGAAG |  |
| Check| fumC R3| TGTTGCTGTAATACAAAG |  |
| fumA | F5     | CAAACAGACTGAGATGGCAGGAGGAGTACTTACATGAGTGGCTGGAGCTGCTTC |  |
| fumA | R3     | GCCGGAACGTTATGCTGAGATG |  |
| fumA | Up R3  | ACATTGTGTTTCTCCTAGATGCTG |  |
| Check| fumA F5| GATGAACCTGAATGGAGAGTG |  |
| Check| fumA R3| CTGGTGGGCTTGGTGAAGT |  |
| mdh  | F5     | TTATCAATAAATAAAGAGAGTTTAGGATGAGTGAGGCTGGAGCTGCTTC |  |
| mdh  | R3     | TTATTCGGCTATGACTTTATTACATGTGAATATGACGTCCATTAG |  |
| mdh  | Down F5| GGTAAATAAGTATGAGGATGCTGGAGCTGCTTC |  |
| Check| mdh F5| TGAAGAAGGCTGAAAATG |  |
| Check| mdh R3| A AgricultGGCATTTACAC |  |
| gltA | F5     | CCAATAAGGGCGCCGCTAAGGAGGACCTTAAATGGGTGTAGGCTGGAGCTGCTTC |  |
| gltA | R3     | ATGGTTACATCCGAGAATATGTTTAAACCATTGAGATATACCTCCTCCTTAG |  |
| gltA | Up R3  | CCACTTAAAGGCTCCTCCTAGCGCCTTATTGG |  |
| check| gltA F5| TCATTGCGGACAGTTATTAG |
| Gene  | Primer 1     | Primer 2     | Sequence                        |
|-------|--------------|--------------|---------------------------------|
| gltA  | R3           |              | CTTCATGGGCTATGAAAAG            |
| ygfH  | F5           |              | CAGTGGACAAGGATGACCGCAGTAGAAGCGGTGTAGGCTGGAGCTGCTTC |
| ygfH  | R3           |              | CATCGAGCCGTTGCAAATTAATTACCGGTGCATATGAATATACCTCCTCTTAG |
| ygfH  | Up R3        |              | CGCTTCATCGGCGGTCTCATCCTTTGCTCCACTG |
| arcA  | F5           |              | TTTAGTGGCATAATTAGGATGCAAAACATGCGTGAGGCTGGAGCTGCTTC |
| arcA  | R3           |              | TGACGGTGGAATACCGGATTAATCTTCACGACTATATGCATATATCCTCCTCTTAG |
| arcA  | Up R3        |              | GCATGTTTGTCACTTAATAGGCAACAACCTGC |
| arcB  | F5           |              | GCAGGGTTGTCCGTGAAAGGATTTCCCCTAATGAGGCTGGAGCTGCTTC |
| arcB  | R3           |              | ACCCAGGTCTAGCCGCCGTCATTTTTAGTCATATGAATATACCTCCTCTCTTAG |
| arcB  | Up R3        |              | TCATTAGGGGAAATTTGCTCACTGCAGCAACACTGC |
| fnr   | F5           |              | ATATCAATTACGGCTTGAGACGACCTATGAGGCTGGAGCTGCTTC |
| fnr   | R3           |              | GTGAGTTTATGCGGAAAAATCACGGCACGATTCTCATATGAATATCCTCCTCTCTTAG |
| fnr   | Up R3        |              | TCATAGGTCTCGTCAGAAGGCTATAGGATAT |
| narH  | F5           |              | AATGATCAGGTACAGGAGAGCGTAAAAATGAGGCTGGAGCTGCTTC |
| narH  | R3           |              | ATGTGAACTAAAATTCGCTTAGTGACGAGGCCATATGAATATCCTCCTCTCTTAG |
| narH  | Up R3        |              | TCATTTCAGCTCTCCTGACCTGATCATT |
| narH  | F5           |              | GGTATCCACACTCCACCTACA          |
| narH  | R3           |              | CAAACGAAATCCCCTATTAAA         |
| adhE  | F5           |              | AAGTTTAAACATTATCAGGAGAGCATTATTGAGGCTGGAGCTGCTTC |
| adhE  | R3           |              | GCCAGACAGCCGTACTGATTTAAGCCTATTTCATATGAATATCCTCCTCTCTTAG |
| adhE  | Up R3        |              | CCATAATCGCTGATGTTAATACCTTTAT |
| adhE  | F5           |              | AGCCACCAATCATACACTACA          |
| adhE  | R3           |              | AAAACCATCTGTTTTTG           |
| nrdD  | F5           |              | CATGTGATAACGACGAGCGCTGCAAAGTGGTGAGGCTGGAGCTGCTTC |
| nrdD  | R3           |              | ATGTGACACCACCTCGATTGCTGCTGCGCCGCATATGAATATCCTCCTCTCTTAG |
| nrdD  | Up R3        |              | CACTTTGCGAGCGCTCTGTTTCATACATG |
| nrdD  | F5           |              | TTGTGATCGATACTACGAA            |
| nrdD  | R3           |              | CAATTTTAAAGTGGTACGAA          |
| tnaA  | F5           |              | TATGTAATGGGAAAATTTAACATTCCTCCTGAGGCTGGAGCTGCTTC  |
| tnaA  | R3           |              | TTTCAAGTTTGCGTGAAGGAGCGCAATATGATATACCTCCTCTTTAG  |
| tnaA  | F5           |              | TATGTAATGGGAAAATTTAACATTCCTCCTGAGGCTGGAGCTGCTTC  |
| tnaA  | R3           |              | TTTCAAGTTTGCGTGAAGGAGCGCAATATGATATACCTCCTCTTTAG  |
| tnaA Up R3 | AGGGAGATGTTTAAGTTTTTCCATTACATA |
| Check tnaA F5 | TCTCATAAACACAGCCAATA |
| Check tnaA R3 | ATACGTGGATTAGCGTGATA |

**Primers used for real time RT-PCR (5’ to 3’)**

| qPCR tnaA F5 | AGGGATTAGAACGCGGTATTG |
| qPCR tnaA R3 | CGGAGTTACTGGTGATGGTTG |
| qPCR dnaJ F5 | ACCAAAGAGATCCGCATTCC |
| qPCR dnaJ R3 | ACGGCAAAGAAACCCTGG |
| qPCR rpoA F5 | GTGACCCTTGAGCCTTTAGAG |
| qPCR rpoA R3 | ACACCATCAATCTCAACCTCG |
Table S5. Proteins with differential expression in the wild-type EHEC strain (EDL933), the isogenic sdhA deletion mutant (EDL933:ΔsdhA), and the sdhA gene complementation strain (EDL933:ΔsdhA-pWF134).

| Protein Name                                | Mass(Da) | EDL933     | ΔsdhA     | t-Test   | Fold Change |
|---------------------------------------------|----------|------------|-----------|----------|-------------|
| Agmatinase                                  | 33557    | 0.84 ± 0.13| 0 ± 0     | 0.0032   | -100        |
| Alcohol dehydrogenase YqhD                  | 42097    | 0.84 ± 0.14| 0 ± 0     | 0.0033   | -100        |
| Aspartate--ammonia ligase                   | 36691    | 1.11 ± 0.12| 0 ± 0     | 0.0008   | -100        |
| Chaperone protein DnaJ                      | 41044    | 1.47 ± 0.32| 0 ± 0     | 0.0103   | -100        |
| Dimethyl sulfoxide reductase DmsA           | 90399    | 1.11 ± 0.29| 0 ± 0     | 0.0193   | -100        |
| Flavodoxin-1                                | 19737    | 1.39 ± 0.05| 0 ± 0     | 1E-05    | -100        |
| GDP-L-fucose synthase                        | 36141    | 0.63 ± 0.02| 0 ± 0     | 0         | -100        |
| Hydrogenase-1 large chain                   | 66253    | 1.18 ± 0.17| 0 ± 0     | 0.0021   | -100        |
| Nitrate/nitrite response regulator protein NarL | 23927  | 0.77 ± 0.15| 0 ± 0     | 0.0075   | -100        |
| Periplasmic nitrate reductase                | 93130    | 0.7 ± 0.06 | 0 ± 0     | 0.0003   | -100        |
| Uncharacterized protein YibN                 | 15596    | 0.7 ± 0.07 | 0 ± 0     | 0.0004   | -100        |
| Uncharacterized protein YniA                 | 32474    | 0.84 ± 0.02| 0 ± 0     | 0         | -100        |
| Succinate dehydrogenase iron-sulfur subunit | 26770    | 4.06 ± 0.37| 0.39 ± 0.39| 0.0024  | -10.31      |
| Succinate dehydrogenase flavoprotein subunit| 64422    | 9.5 ± 0.23 | 1.01 ± 1.01| 0.0011  | -9.45       |
| Xaa-Pro aminopeptidase                       | 49815    | 1.18 ± 0.13| 0.22 ± 0.22| 0.0179  | -5.49       |
| Universal stress protein E                   | 35707    | 2.22 ± 0.36| 0.52 ± 0.27| 0.0190  | -4.24       |
| Osmotically-inducible protein Y              | 21074    | 1.39 ± 0.17| 0.43 ± 0.22| 0.0246  | -3.22       |
| Protein                             | Accession No. | Ratio 3/1 | p-Value 3/1 | Fold Change |
|------------------------------------|---------------|-----------|-------------|-------------|
| Protein HemY                       | 45245         | 1.82 ± 0.09 | 0.81 ± 0.14 | 0.0034 | -2.24 |
| Chaperone protein skp              | 17688         | 2.45 ± 0.19 | 1.21 ± 0.36 | 0.0392 | -2.03 |
| Fumarate reductase iron-sulfur subunit | 27123       | 2.24 ± 0.3  | 1.19 ± 0.17 | 0.0370 | -1.88 |
| 30S ribosomal protein S12          | 13737         | 4.41 ± 0.67 | 2.38 ± 0.12 | 0.0404 | -1.86 |
| Protein YdgH                       | 33903         | 2.59 ± 0.26 | 1.4 ± 0.3   | 0.0389 | -1.85 |
| Tryptophanase                     | 52773         | 37.08 ± 2.43 | 20.99 ± 2.55 | 0.0102 | -1.77 |
| Cystine-binding periplasmic protein | 29039       | 1.54 ± 0.18 | 0.89 ± 0.03 | 0.0247 | -1.72 |
| 3-mercaptopyruvate sulfurtransferase | 30826       | 1.54 ± 0.06 | 0.9 ± 0.15  | 0.0186 | -1.71 |
| 50S ribosomal protein L13         | 16019         | 9.35 ± 0.4  | 7.87 ± 0.28 | 0.0374 | -1.19 |
| Molecular chaperone Hsp31 and glyoxalase 3 | 31220     | 1.05 ± 0.02 | 0.89 ± 0.03 | 0.0080 | -1.17 |

**Up regulation in ΔsdhA VS. EDL933**

| Protein                                      | Accession No. | Ratio 3/1 | p-Value 3/1 | Fold Change |
|----------------------------------------------|---------------|-----------|-------------|-------------|
| Inosine-5′-monophosphate dehydrogenase       | 52022         | 4.68 ± 0.07 | 5.41 ± 0.13 | 0.0071 | 1.16 |
| Aconitate hydratase 2                        | 93498         | 21.29 ± 0.24 | 24.85 ± 0.28 | 0.0006 | 1.17 |
| 2-oxoglutarate dehydrogenase E1 component    | 105062        | 20.31 ± 0.41 | 25.38 ± 1.66 | 0.0409 | 1.25 |
| Phosphopentomutase                           | 44370         | 6.21 ± 0.1  | 7.92 ± 0.49 | 0.0271 | 1.27 |
| Phosphate acetyltransferase                  | 77172         | 9.57 ± 0.13 | 12.38 ± 0.91 | 0.0383 | 1.29 |
| Long-chain fatty acid transport protein       | 48539         | 2.11 ± 0.46 | 3.95 ± 0.37 | 0.0361 | 1.87 |
| Putative uncharacterized protein             | 29257         | 1.54 ± 0.18 | 3.11 ± 0.06 | 0.0011 | 2.03 |
| Flagellin                                    | 51295         | 5.09 ± 0.54 | 10.98 ± 1.25 | 0.0123 | 2.16 |
| Flagellin (Fragment)                         | 56672         | 3.38 ± 1.48 | 7.77 ± 0.54 | 0.0497 | 2.3 |
| 50S ribosomal protein L20                    | 13497         | 0.21 ± 0.21 | 2.3 ± 0.43  | 0.0121 | 11.11 |
| Phosphomannomutase                           | 50340         | 0 ± 0      | 1.25 ± 0.37 | 0.0277 | 100 |
## Up regulation in ΔsdhA-pWF134 vs. ΔsdhA

| Protein Name                                      | Gene ID | Fold Change | p-value | Percentage |
|---------------------------------------------------|---------|-------------|---------|------------|
| Ampicillin resistance protein                     | 31557   | 0 ± 0       | 1.23 ± 0.14 | 0.0008     | 100        |
| Beta-galactosidase                                | 116462  | 0 ± 0       | 2.14 ± 0.49 | 0.0120     | 100        |
| Beta-lactamase                                    | 31515   | 0 ± 0       | 0.87 ± 0.12 | 0.0018     | 100        |
| Beta-lactamase TEM                                | 31515   | 0 ± 0       | 8 ± 0.38   | 3E-05      | 100        |
| Chaperone protein DnaJ                            | 41044   | 0 ± 0       | 1.51 ± 0.16 | 0.0007     | 100        |
| Protein dcrB                                      | 19787   | 0 ± 0       | 1.29 ± 0.41 | 0.0347     | 100        |
| Putative acyl-CoA thioester hydrolase ybhC        | 46082   | 0 ± 0       | 0.79 ± 0.04 | 5E-05      | 100        |
| Succinate dehydrogenase                           | 26784   | 0 ± 0       | 6.18 ± 1.21 | 0.0069     | 100        |
| Succinate dehydrogenase flavoprotein subunit      | 64422   | 1.01 ± 1.01 | 71.48 ± 16.92 | 0.0141     | 70.92      |
| Succinate dehydrogenase iron-sulfur subunit       | 26770   | 0.39 ± 0.39 | 26 ± 1.98  | 0.0002     | 65.79      |
| Lactaldehyde dehydrogenase                        | 52273   | 1.03 ± 0.62 | 2.96 ± 0.18 | 0.0414     | 2.86       |
| Tryptophanase                                     | 52773   | 20.99 ± 2.55 | 47.72 ± 4.72 | 0.0075     | 2.27       |
| Acriflavine resistance protein A                   | 42197   | 2.22 ± 0.08 | 3.75 ± 0.39 | 0.0186     | 1.69       |
| Adenylosuccinate synthetase                       | 47345   | 6.33 ± 0.82 | 10.66 ± 0.72 | 0.0163     | 1.68       |
| Chaperone protein DnaK                            | 69115   | 27.4 ± 2.12 | 38.29 ± 2.53 | 0.0300     | 1.4        |
| Chaperone protein HtpG                            | 71449   | 14.15 ± 0.23 | 19.75 ± 1.98 | 0.0482     | 1.4        |
| Protein GrpE                                      | 21741   | 2.3 ± 0.08  | 2.83 ± 0.09 | 0.0128     | 1.23       |

## Down regulation in ΔsdhA-pWF134 VS. ΔsdhA

| Protein Name                                      | Gene ID | Fold Change | p-value | Percentage |
|---------------------------------------------------|---------|-------------|---------|------------|
| Pyruvate dehydrogenase E1 component               | 99668   | 26.62 ± 0.08 | 23.11 ± 0.94 | 0.0203     | -1.15      |
| Aerobic respiration control protein ArcA           | 27292   | 4.31 ± 0.21 | 3.16 ± 0.31 | 0.0390     | -1.36      |
| Protein Name                                      | Accession Number | Fold Change | p-value | Z-score |
|--------------------------------------------------|------------------|-------------|---------|---------|
| DNA-directed RNA polymerase subunit beta         | 150632           | 37.4 ± 2.31 | 0.0349  | -1.37   |
| Peroxiredoxin OsmC                               | 15088            | 2.3 ± 0.08  | 0.0185  | -1.38   |
| Transcriptional regulatory protein OmpR          | 27354            | 3.32 ± 0.39 | 0.0454  | -1.53   |
| 2-oxoglutarate dehydrogenase E1 component       | 105062           | 25.38 ± 1.66| 0.0057  | -1.56   |
| Uncharacterized protein YggE                     | 26635            | 1.48 ± 0.16 | 0.0323  | -1.57   |
| Aconitase hydratase 1                            | 97677            | 14.08 ± 0.66| 0.0312  | -1.63   |
| Glutamate decarboxylase alpha                    | 52699            | 98.17 ± 8.26| 0.0352  | -1.88   |
| Mannose-1-phosphate guanylyltransferase 2        | 54270            | 3.05 ± 0.22 | 0.0122  | -2.04   |
| Long-chain fatty acid transport protein          | 48539            | 3.95 ± 0.37 | 0.0148  | -2.1    |
| Biosynthetic arginine decarboxylase              | 73886            | 2.43 ± 0.33 | 0.0361  | -2.28   |
| Flagellin                                        | 51295            | 10.98 ± 1.25| 0.0048  | -3.91   |
| Probable phospholipid-binding protein MlaC       | 23963            | 2.45 ± 0.39 | 0.0231  | -4.04   |
| Glutaminase 1                                    | 32844            | 0.88 ± 0.11 | 0.0482  | -4.15   |
| HTH-type transcriptional regulator IscR          | 17337            | 1.12 ± 0.16 | 0.0272  | -5.27   |
| Flagellin (Fragment)                             | 56672            | 7.77 ± 0.54 | 0.0004  | -5.69   |
| Cyclopropane-fatty-acyl-phospholipid synthase    | 43777.81         | 1.12 ± 0.06 | 0 ± 0   | -100    |
| Lysine-arginine-ornithine-binding periplasmic protein | 27992       | 1.33 ± 0.23 | 0 ± 0   | -100    |
| Protein phosphatase CheZ                         | 23976            | 1.9 ± 0.5   | 0.0184  | -100    |
| GO category | Total gene/hit number | Gene Name | Segment Type | Primary Hit | \( P \) value of liquid-based survival (EDL933 vs mutants) | \( P \) value of agar-based survival (EDL933 vs mutants) | Product or Function |
|-------------|----------------------|-----------|--------------|-------------|----------------------------------------------------------|----------------------------------------------------------|-------------------|
| Metabolism  | 26/32                | sdhA      | backbone     | ED97-A-1    | < 0.0001                                                | < 0.0001                                                | succinate dehydrogenase, flavoprotein subunit; Energy metabolism, carbon: TCA cycle |
|             |                      | sdhC      | backbone     | ED201-E-9   | < 0.0001                                                | < 0.0001                                                | succinate dehydrogenase, cytochrome b556; Energy metabolism, carbon: TCA cycle |
|             |                      | gltA/sdhC | backbone     | ED29-D-11, ED54-D-6 | < 0.0001 (ED54-D-6) | < 0.0001 (ED29-D-11) | type II citrate synthase / succinate dehydrogenase, cytochrome b556; Energy metabolism, carbon: TCA cycle |
|             |                      | mdh      | backbone     | ED61-A-7, ED61-C-11 | < 0.0001, < 0.0001 | growth defect | malate dehydrogenase; Energy metabolism, carbon: TCA cycle |
|             |                      | manB     | 84 O-Island  | ED56-G-9    | < 0.0001                                                | < 0.0001                                                | phosphomannomutase; Central intermediary metabolism |
|             |                      | treC      | backbone     | ED38-B-9    | < 0.0001                                                | 0.4107                                                  | mreB 6-F hydrolase; Degradation of small molecules: Carbon compounds |
|             |                      | bioH      | backbone     | ED185-D-2   | 0.0873                                                  | < 0.0001                                                | biotin biosynthesis; Biosynthesis of cofactors, carriers: Biotin |
|             |                      | fbp       | backbone     | ED1-A-7     | < 0.0001                                                | 0.0929                                                  | fructose-bisphosphatase; Central intermediary metabolism: Gluconeogenesis |
|             |                      | nuoE      | backbone     | ED135-E-12  | < 0.0001                                                | 0.7054                                                  | NADH dehydrogenase I chain I; Energy metabolism, carbon: Aerobic respiration |
|             |                      | nuoB      | backbone     | ED208-B-9   | < 0.0001                                                | 0.0232                                                  | NADH dehydrogenase I chain B; Energy metabolism, carbon: Aerobic respiration |
|             |                      | nuoH      | backbone     | ED196-G-6   | < 0.0001                                                | < 0.0001                                                | NADH dehydrogenase I chain H; Energy metabolism, carbon: Aerobic respiration |
|             |                      | nuoM      | backbone     | ED207-F-4   | < 0.0001                                                | 0.2743                                                  | NADH dehydrogenase I chain M; Energy metabolism, carbon: Aerobic respiration |
|             |                      | nuoG      | backbone     | ED203-B-3   | < 0.0001                                                | 0.0294                                                  | NADH dehydrogenase I chain G; Energy metabolism, carbon: Aerobic respiration |
|             |                      | atpI      | backbone     | ED86-H-8    | < 0.0001                                                | growth defect | membrane-bound ATP synthase; ATP-proton motive force interconversion |
|             |                      | atpD      | backbone     | ED30-F-7    | < 0.0001                                                | growth defect | membrane-bound ATP synthase, F1 sector, beta-subunit; ATP-proton motive force interconversion |
|             |                      | araA      | backbone     | ED1-F-12    | < 0.0001                                                | 0.003                                                   | 5-enolpyruvylshikimate-3-phosphate synthetase; Amino acid biosynthesis: Chorismate |
|             |                      | thrB      | backbone     | ED139-D-5   | < 0.0001                                                | auxotroph | homoserine kinase; Amino acid biosynthesis: Threonine |
|             |                      | pfs       | backbone     | ED127-G-8   | < 0.0001                                                | 0.0002                                                  | orf, hypothetical protein; Unknown function, 5'-methylthioadenosine-5'-adenosylomycocystein nucleosidase |
|             |                      | udhA      | backbone     | ED138-B-4, ED205-G-12, ED32-B-6 | < 0.0001, < 0.0001, < 0.0001 | 0.0370 (ED138-B-4), 0.0468 (ED205-G-12) | putative oxidoreductase; Not classified |
|             |                      | ubiE      | backbone     | ED137-C-6   | < 0.0001                                                | growth defect | Biosynthesis of cofactors, carriers: Menaquinone, ubiquinone |
|             |                      | epd       | Blackbone    | ED160-C-11  | < 0.0001                                                | 0.5628                                                  | D-erythrose 4-phosphate dehydrogenase; Central intermediary metabolism |
|             |                      | gnd       | backbone     | ED143-B-7, ED184-D-5 | < 0.0001, < 0.0001 | < 0.0001 (ED143-B-7), < 0.0001 (ED184-D-5) | glutamate-6-phosphate dehydrogenase, decarboxylating |
|             |                      | pnp       | backbone     | ED52-F-4    | < 0.0001                                                | < 0.0001                                                | polynucleotide phosphorylase; cytidylate kinase activity; Macromolecule synthesis, modification: RNA synthesis, modification, DNA transcription |
|             |                      | pta       | backbone     | ED194-E-2   | < 0.0001                                                | 0.1578                                                  | phosphotransacetylase; Degradation of small molecules: Carbon compounds |
|             |                      | guaA      | backbone     | ED86-A-10, ED74-A-9 | < 0.0001, < 0.0001 | auxotroph | GMP synthetase (glutamine-hydrolyzing); Nucleotide biosynthesis: Purine ribonucleotide biosynthesis |
|             |                      | bioC      | backbone     | ED134-E-11  | < 0.0001                                                | < 0.0001                                                | biotin biosynthesis; reaction prior to pimelykol CoA |
| Lipopolysaccharide |                        | rfaD      | 145 O-Island  | ED46-C-7    | < 0.0001                                                | < 0.0001                                                | ADP-L-glycerol-3-mannoheptose-6-epimerase; Cell exterior constituents: Surface polysaccharides and antigens |
|             |                      | rfaG      | Hypermutable | ED55-A-5, ED132-A-10, ED189-B-9, ED172-D-12 | < 0.0001, < 0.0001, < 0.0001, < 0.0001, < 0.0001 | < 0.0001 (ED55-A-3) | glucosyltransferase I; lipopolysaccharide core biosynthesis |
|             |                      | rfaC      | Junction     | ED122-G-3, ED203-A-4 | < 0.0001, < 0.0001 | < 0.0001, < 0.0001 | heptosyl transferase I; lipopolysaccharide core biosynthesis |
|             |                      | rfaF      | backbone     | ED74-D-7    | < 0.0001                                                | < 0.0001                                                | ADP-heptose-6-p-lps heptosyltransferase II; lipopolysaccharide core biosynthesis |
|             |                      | Z4405     | backbone     | ED196-F-4   | < 0.0001                                                | < 0.0001                                                | putative kinase; Not classified, RfaE-like |
|             |                      | waaI      | 145 O-Island  | ED34-B-1, ED34-G-10, ED58-D-3, ED51-A-5 | < 0.0003 (ED34-G-10), < 0.0001 (ED58-D-3) | < 0.0001 (ED34-B-1), < 0.0001 (ED34-G-10) | putative LPS biosynthesis enzyme; Cell exterior constituents: Surface polysaccharides and antigens |
|             |                      | waaP      | Junction     | ED82-D-12, ED82-G-1, ED68-H-7, ED45-C-10 | < 0.0001, < 0.0001, < 0.0001, < 0.0001 | < 0.0001 (ED82-D-12), 0.2475 (ED62-G-1) | putative LPS biosynthesis enzyme; Cell exterior constituents: Surface polysaccharides and antigens |
| & Cell exterior constituents biosynthesis | 15/33 | wcy & 84 O-Island & ED156-G-2, ED62-G-3, ED185-E-2 | < 0.0001, < 0.0001, 0.0005 | 0.0255 (ED156-G-2), 0.5712 (ED62-G-3) | O antigen polymerase; Cell exterior constituents: Surface polysaccharides and antigens |
| & ManC & 84 O-Island & ED190-D-5, ED137-E-4 | < 0.0001, < 0.0001 | 0.0001 (ED137-E-4) | mannose-1-P guanylyltransferase; Cell exterior constituents: Surface polysaccharides and antigens |
| per & 84 O-Island & ED186-D-7, ED206-H-5, ED208-E-11 | < 0.0001, 0.0009, < 0.0001 | < 0.0001 (ED186-D-7) | perosamine synthetase; Cell exterior constituents: Surface polysaccharides and antigens |
| wbdP & 84 O-Island & ED68-E-1, ED130-A-11, ED105-H-5 | < 0.0001, < 0.0001, < 0.0001 | < 0.0001 (ED68-E-1) | glycosyl transferase; Cell exterior constituents: Surface polysaccharides and antigens |
| csgB & backbone & ED232-I-9 | < 0.0001 | 0.018 | minor csgB subunit precursor, similar to CsgA; Cell exterior constituents: Surface structures |
| fcI & 84 O-Island & ED134-E-4 | < 0.0001 | < 0.0001 | fucose synthetase; Cell exterior constituents: Surface polysaccharides and antigens |
| Z3198 & 84 O-Island & ED12-E-8, ED151-I-8 | < 0.0001, < 0.0001 | < 0.0001 (ED12-E-8), 0.0002 (ED151-I-8) | GDP-mannose dehydratase; Cell exterior constituents: Surface polysaccharides and antigens |
| wbdR & 84 O-Island & ED155-D-11 | < 0.0001 | 0.0032 | acetyl transferase; Cell exterior constituents: Surface polysaccharides and antigens |

| Type three secretion system | 4/4 | eae & 148 O-Island & ED184-F-2 | < 0.0001 | < 0.0001 | intimin adherence protein; Extracellular functions: Secreted proteins |
| Z2240/Z2241 & 62 O-Island/Junction & ED52-B-8 | < 0.0001 | 0.5175 | hypothetical protein/hypothetical protein, putative type III effector protein, T3SS effector-like protein EspW-homolog |
| Z3919/Z3920 & 108 O-Island & ED38-H-5 | 0.0014 | 0.002 | hypothetical protein/hypothetical protein, non-LEE-encoded type III effector, T3SS secreted effector EspW-like protein |
| ler & 148 O-Island & ED77-C-1 | 0.0005 | 0.6197 | orf, hypothetical protein; Unknown function; DNA-binding protein H-NS, transcriptional regulator Ler-like |
| ompr & 84 O-Island & backbone & ED14-C-5, ED176-A-4 | < 0.0001, < 0.0001 | < 0.0001 | response regulator (sensor, EnvZ) affecting transcription of ompC, and ompF; outer membrane protein synthesis; Global regulatory functions |
| Z3603 & backbone & ED4-B-11 | < 0.0001 | 0.9179 | orf, hypothetical protein; Unknown function; phosphohistidine phosphatase |

| DNA Recombination & Repair | 3/3 | rvsC & backbone & ED198-E-1 | < 0.0001 | < 0.0001 | Holliday junction nucleases; resolution of structures; Macromolecule synthesis, modification |
| xerD & backbone & ED144-E-6 | < 0.0001 | 0.0002 | site-specific recombinase; Macromolecule synthesis, modification: DNA-replication, repair, modification |
| Z1201 & 43 O-Island & ED201-H-5 | < 0.0001 | 0.0517 | orf, Unknown function, EHEC-specific, UvrD/REP helicase-like protein |

| Transport protein | 3/3 | tolQ & backbone & ED52-E-9 | < 0.0001 | < 0.0001 | inner membrane protein, membrane-spanning, maintains integrity of cell envelope; tolerance to group A colicins; Colicin-related functions |
| tolA & Junction & ED198-C-3 | < 0.0001 | < 0.0001 | membrane spanning protein, required for outer membrane integrity; Colicin-related functions |
| betT & backbone & ED201-A-1 | < 0.0001 | 0.0322 | high-affinity choline transport; Transport of small molecules: Other |

| Cell Protection Systems | 3/3 | cunC & backbone & ED203-G-2 | < 0.0001 | 0.0139 | copper homeostasis protein; Protection responses: Detoxification |
| yhaA & backbone & ED16-F-11 | < 0.0001 | < 0.0001 | putative enzyme; Not classified, a predicted cytochrome c peroxidase |
| hdcA & backbone & ED159-G-6 | < 0.0001 | < 0.0001 | orf, hypothetical protein; Unknown function, stress response protein acid-resistance protein; |
| hfg & backbone & ED185-H-8 | < 0.0001 | 0.0279 | host factor I for bacteriophage Q beta replication, a growth-related protein; unknown function |
| yhgA & backbone & ED15-D-2 | 0.0029 | < 0.0001 | orf, hypothetical protein; Unknown function, transposase |
| yheO & backbone & ED51-C-3 | < 0.0001 | 0.5491 | orf, hypothetical protein; Unknown function, DNA-binding transcriptional regulator |
| ydeK & backbone & ED121-D-5 | < 0.0001 | 0.0099 | orf, Hypothetical protein; Unknown function, putative lipoprotein/auto transporter; Extended Signal Pediants of Type V secretion system |
| yiaF & backbone & ED141-F-8 | < 0.0001 | 0.2029 | orf, Hypothetical protein; Unknown function, putative outer membrane lipoprotein |
| Z1205 & 43 O-Island & ED207-F-11 | < 0.0001 | < 0.0001 | orf, Unknown function, EHEC-specific |
| Z2973 & 76 O-Island/Junction & ED200-A-11 | < 0.0001 | < 0.0001 | unknown protein encoded by prophage CP-333T, EHEC-specific |
| Z2256/Z2257 & 46 O-Island/Junction & ED163-E-10 | < 0.0001 | < 0.0001 | Rhs element protein/Rhs element protein, Rhs family protein [Cell envelope biogenesis, outer membrane] |
| ZD406/Z0407 & backbone & ED1-A-9 | < 0.0001 | 0.0205 | hypothetical protein/putative transcription factor, ANK; ankyrin repeats; ankyrin repeats mediate protein-protein interactions |
| yagU & backbone & ED82-G-12 | < 0.0001 | auxotroph | orf, Unknown function |

| Total | 66/91 |
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