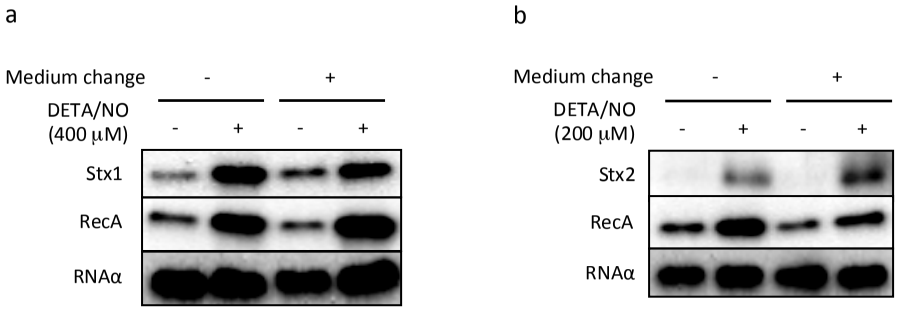
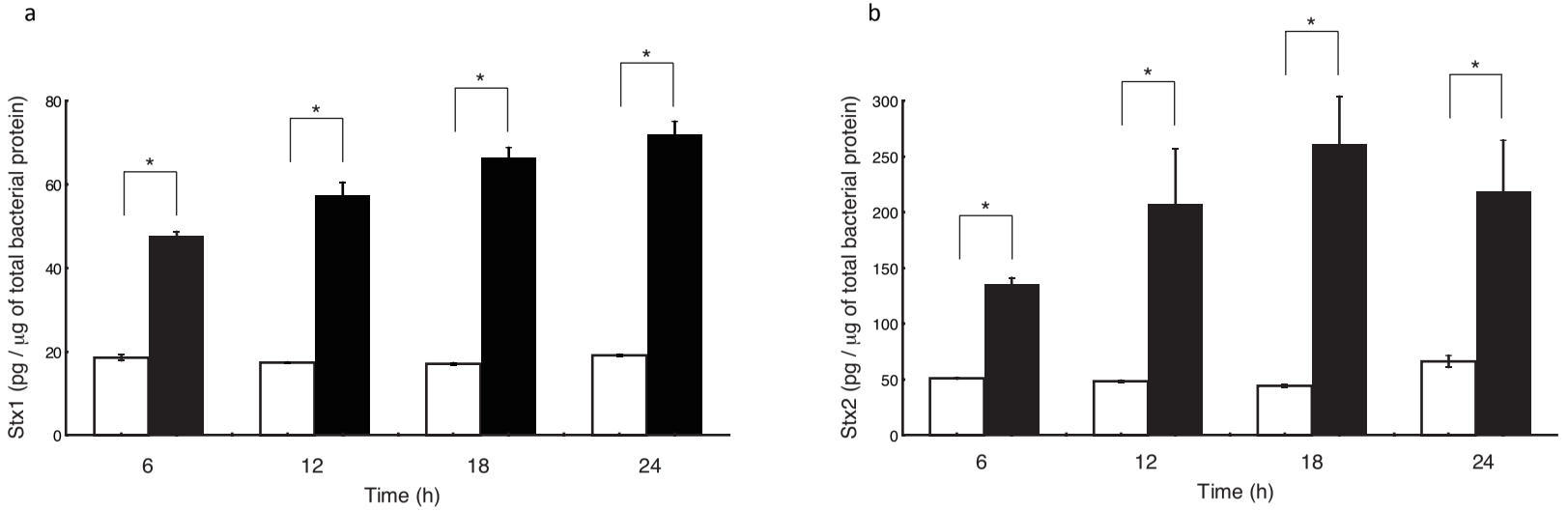
Fig. S1



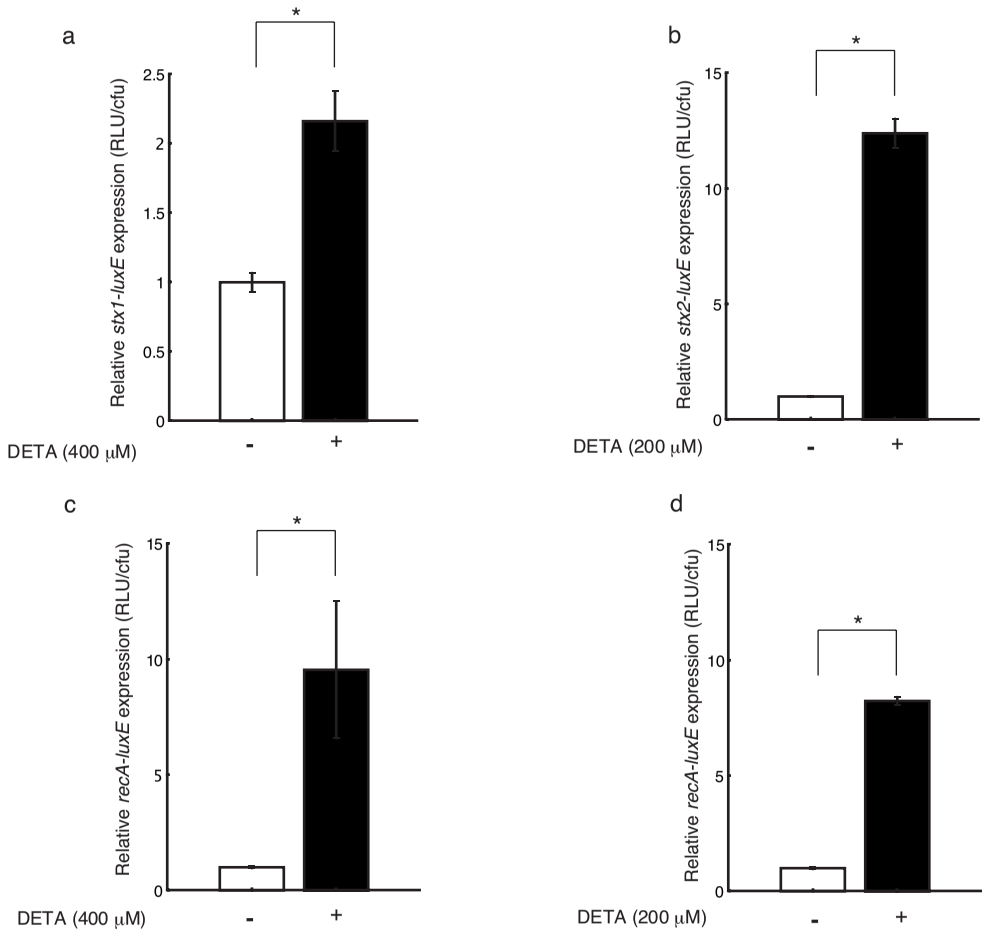

Supplemental Figure 1
The enhancements of Shiga toxin production and RecA expression in the deleted *norV*-type EHEC are sufficient for exposure to NO at the log phase.
EHEC EDL933 grown overnight was diluted with LB broth containing DETA-NONOate (DETA/NO) and then grown statically at 37°C for 18 h under anaerobic conditions as a control. After 6 h the cells were washed, and medium was changed to LB broth without an NO donor. EHEC were further incubated for 12 h at 37°C under anaerobic conditions.
(a) EHEC strains were fractionated into cell-associated fractions. A 0.2 μg sample of each protein of the cell-associated fraction was analyzed by immunoblot analysis using anti-Stx1 antiserum, anti-RecA antibody and anti-RNA α antibody as an internal control.
(b) EHEC strains were fractionated into the cell-associated fractions and the culture supernatant fractions. A 0.2 μg sample of each protein of the cell-associated fraction was analyzed by immunoblot analysis using anti-RecA antibody and anti-RNA α antibody as an internal control. Each volume, which corresponds to 0.2 μg of protein of the cell-associated fraction, of the supernatant fraction was analyzed by immunoblot analysis using anti-Stx2 antiserum.

Fig. S2



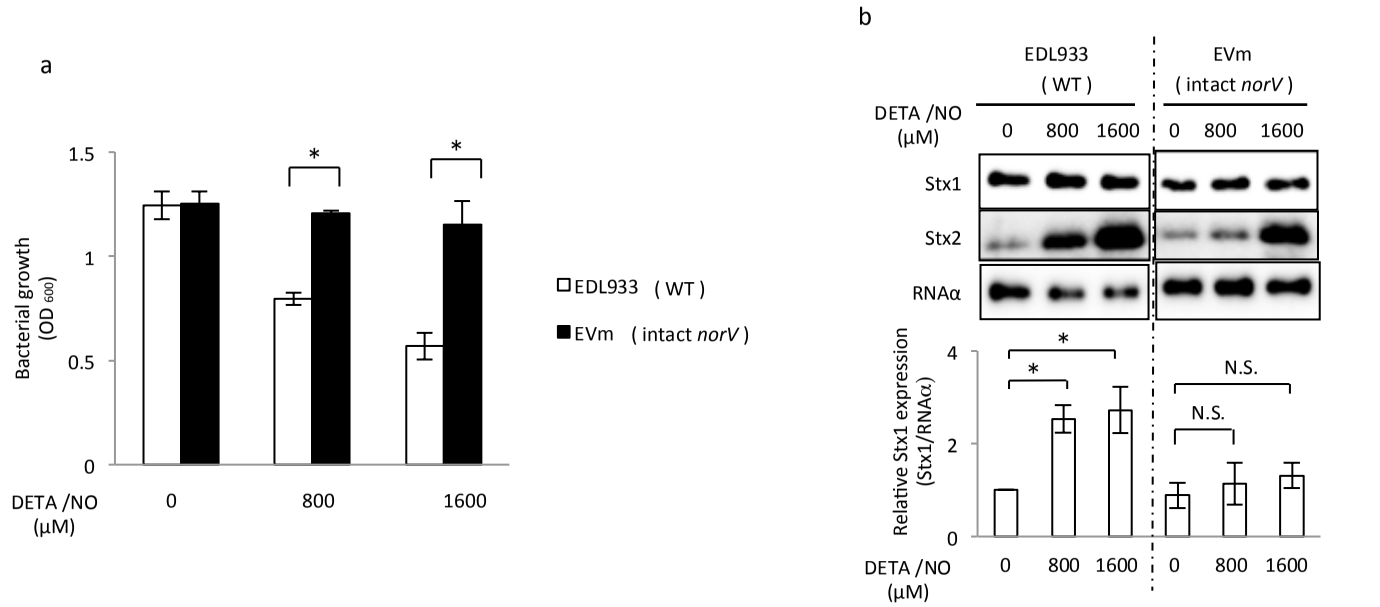

Supplemental Figure 2
NO enhances the production of Shiga toxins in the deleted *norV*-type EHEC under anaerobic conditions.
EHEC EDL933 were cultured in LB medium containing various concentrations of DETA-NONOate (DETA/NO) at 37°C under anaerobic conditions. EHEC strains were fractionated into cell-associated fractions and supernatant fractions at the indicated times. The amount of Stx1 in the cell-associated fraction (a) and the amount of Stx2 in the supernatant fraction (b) were measured by Gb3-ELISA. The amounts of total proteins in the cell-associated fraction were determined by Bradford method.
Data are the means ± standard deviations of values from three experiments. *$p < 0.01$.

Fig. S3



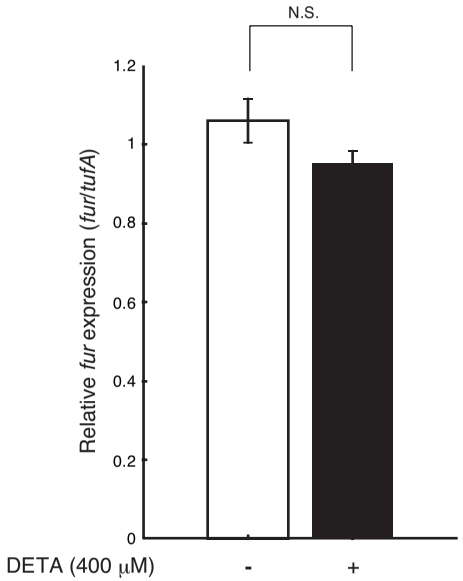

Supplemental Figure 3
NO enhances transcriptions of *stx1*, *stx2* and *recA* in the deleted *norV*-type EHEC under anaerobic conditions.
EHEC reporter strains were cultured in LB medium containing various concentrations of DETA-NONOate (DETA/NO) for 18 h at 37°C under anaerobic conditions.
Relative light units (RLU) and the number of bacteria were measured by a luminometer and bacteria plate counts (cfu), respectively.
(a) *stx1*-reporter E1-E2S (pluxCDAB3) treated with or without 400 μM DETA/NO.
(b) *stx2*-reporter E(SR)2-E1S (pluxCDAB3) treated with or without 200 μM DETA/NO.
(c) *recA*-reporter ERSA (pluxCDAB3) treated with or without 400 μM DETA/NO.
(d) *recA*-reporter ERSA (pluxCDAB3) treated with or without 200 μM DETA/NO.
Data are the means ± standard deviations of values from three experiments. *$p < 0.01$.

Fig. S4



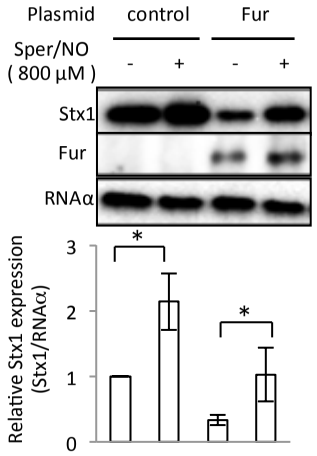

Supplemental Figure 4
Role of intact NorV in NO-mediated aerobic growth inhibition and Shiga toxin production in EHEC under aerobic conditions.
EHEC strains grown overnight were diluted with LB medium containing various concentrations of DETA-NONOate (DETA/NO) and grown statically for 18 h at 37°C under aerobic conditions.
(a) The optical density at 600 nm ($OD_{600}$) was determined. Data are the means ± standard deviations of values from four experiments. *$p < 0.01$.
(b) EHEC strains were fractionated into culture supernatant fractions and cell-associated fractions. A 0.2 μg sample of each protein of the cell-associated fraction was analyzed by immunoblot analysis using anti-Stx1 antiserum and anti-RNA α antibody as an internal control. Each volume, which corresponds to 0.2 μg of protein of the cell-associated fraction, of the supernatant fraction was analyzed by immunoblot analysis using anti-Stx2 antiserum. The relative amounts of Stx1 and Stx2 were quantified by densitometry and normalized to internal control RNA α. Data are the means ± standard deviations of values from four experiments. *$p < 0.01$; N.S. , not significant.

Fig. S5




Supplemental Figure 5
Effect of NO on *fur* expression in the deleted *norV*-type EHEC under anaerobic conditions. EHEC strains grown overnight were diluted with LB medium containing 400 μM DETA-NONOate (DETA/NO) and grown statically for 6 h at 37°C under aerobic conditions. The expression level of *fur* mRNA in the deleted *norV*-type EHEC was analyzed by real-time RT-PCR. Data are the means ± standard deviations of values from three experiments. N.S. , not significant.

Fig. S6




Supplemental Figure 6
Effect of Fur overexpression on NO-enhanced Stx1 production in EHEC under anaerobic conditions. EHEC EDL933 harboring the Fur expression plasmid (pTrcHis2A-fur) or a control plasmid (pTrcHis2A) were cultured in LB medium containing 800 μM NO donor Spermine NONOate (Sper/NO) with or without 1 mM IPTG for 18 h at 37°C under anaerobic conditions. EHEC strains were fractionated into cell-associated fractions. The samples were analyzed by immunoblot analysis using anti-Stx1 antiserum, anti-His Tag antibody for detection of Fur-His and anti-RNA α antibody as an internal control. The relative amounts of Stx1 were quantified by densitometry and normalized to internal control RNA a. Data are the means ± standard deviations of values from four experiments. *$p < 0.01$.

Supplemental Table S1　PCR primers

| Primer | Sequence | Reference |
|---|---|---|
| P16 | GTGGCTGGGAAGGTACTCG | Svensson *et al.* (2010) |
| P17 | AGCCAGACGCCGAGATATTG | Svensson *et al.* (2010) |
| P600 | GACCGAGGTAGCCAGAATACTGAACTGTAGTCGCAACACGGTCAGAAAATTAACCCTCACTAAAGGGCG | This study |
| P613 | TCTACGAGTTTGCCAGCCTCCCCCAGTGGCTGGCTTTTTTATGTCCGTAATTAACCCTCACTAAAGGGCG | Shimizu *et al.* (2009) |
| P628 | CCACCCCCTGAAGGACGGCGTTTTACGGCGCACCGGATAAACGTAACTAATACGACTCACTATAGGGCTC | Shimizu *et al.* (2009) |
| P715 | GGCCATGGCCGCTTAGTAACAGGAC | This study |
| P724 | ATTAACAATATTTGCCAGGGACTTGTGGTTTTCATTTAGGCGTGGCAATTAACCCTCACTAAAGGGCG | This study |
| P725 | ACAAATAAGTGAGAGCTGTAACTCTCGCTTTTCTTATTTCCCTTGCATAATACGACTCACTATAGGGCTC | This study |
| P845 | TACAACTTAAAAAGCAAAAGGGCCGCAGATGCGACCCTTGTGTATCAAATACGACTCACTATAGGGCTC | This study |
| P846 | TCGCTGCGCCGTTTAAA | This study |
| P890 | TGGTTGATGACGAAGAGCTG | Vareille *et al.* (2007) |
| P891 | GCTCTGGTTCCGGAATGTAA | Vareille *et al.* (2007) |
| P1049 | CCCAGCTGCCTGTGTATCAATAAATGTT | This study |
| P1050 | GGCCATGGATTACACAATACTCCTTGAG | This study |
| P1064 | GGGTTAAGCAGCGTGGCAATGTAACCACTCTTATCATGATATGCAGAAATACGACTCACTATAGGGCTC | This study |
| P1077 | GGGCGGCCGCGATCAGGTCGCGGTAAT | This study |
| P1078 | GGGCGGCCGCAAGACGATTAAAAATCTTCGTT | This study |
| P1079 | CAATTTTATAAAGGCTCCATCATG | This study |
| P1080 | GCCTTTATAAAATTGTTTCTCAAT | This study |
| P1081 | GGTAAATACTCCATCATGCGCCTG | This study |
| P1082 | GATGGAGTATTTACCAAATTGTTT | This study |
| P1085 | ACCGGCGCGCAGGCACTGGAAATC | This study |
| P1086 | TGCCTGCGCGCCGGTGTCCGGCTG | This study |
| P1089 | ATGTTCAGTAACCCGGAAACCACT | This study |
| P1090 | CGGGTTACTGAACATCACACCAAT | This study |
| P1093 | CGTCTCGCCATCCGTCGTATCGGC | This study |
| P1094 | ACGGATGGCGAGACGAACAGAGGC | This study |
| P1095 | AAAGCATACGCGTGGTACAGCTAC | This study |
| P1096 | CCACGCGTATGCTTTCTCGATCAG | This study |
| P1100 | GCGATCAGGTCGCGGTAAT | This study |
| P1215 | TTTGAATGGAATTATCATTTTCATTAC | This study |
| P1216 | ATAATTCCATTCAAAGAGAGCTGCAA | This study |
| P1243 | GTATCGTCACCCGCCACAA | Yang *et al.* (2015) |
| P1244 | CGCAGTGACCGTAAAGATAGAGA | Yang *et al.* (2015) |
| P10011 | GGGTCGACTTTGCCTTCGTGCGCGT | This study |
| P10002 | AACAGAACATATTGACTATCCGGTATTACCCGGCATGACAGGAGTAAAATTAACCCTCACTAAAGGGCG | This study |

References


Svensson, L., Poljakovic, M., Save, S., Gilberthorpe, N., Schon, T., Strid, S., … Persson, K. (2010). Role of flavohemoglobin in combating nitrosative stress in uropathogenic *Escherichia coli*--implications for urinary tract infection. Microb pathog 49: 59–66.

Yang, B., Feng, L., Wang, F. and Wang, L. (2015). Enterohemorrhagic *Escherichia coli* senses low biotin status in the large intestine for colonization and infection. Nat Commun 6: 6592.

Supplemental Table S2　Construction of template *recA*-mutated plasmid

| Mutant plasmid | PCR primers | Template plasmid |
|---|---|---|
| precAG22Y1 | P1079-P1080 | pFRT-recA2 |
| precAG24Y1 | P1081-P1082 | pFRT-recA2 |
| precAE123A3 | P1093-P1094 | pFRT-recA2 |
| precAG204S3 | P1095-P1096 | pFRT-recA2 |
| precAD224A1 | P1089-P1090 | pFRT-recA2 |
| precAG288Y1 | P1085-P1086 | pFRT-recA2 |

Supplemental Table S3　Construction of isogenic mutant strains

| Mutant strain | PCR primers | Template plasmid | Parent strain | Target locus | Kan cassette |
|---|---|---|---|---|---|
| ERSA | P845-P846 | pLCE19 | EDL933 | downstream of *recA* | - |
| E1Q1 | P1064-P600 | pFRT-kan | EDL933 | *Q* of Stx1-phage | - |
| EDLf | P724-P725 | pFRT-kan | EDL933 | *fur* | - |
| ERA1-1 | P10002-P845 | pFRT-Kan | EDL933 | *recA* | - |
| EP | P613-P628 | pFRT-Kan | EDL933 | *stx1* promotor | - |
| ERG22Y | P1100-P845 | precAG22Y1 | ERA1-1 | *recA* | - |
| ERG24Y | P1100-P845 | precAG24Y1 | ERA1-1 | *recA* | - |
| ERE123A | P1100-P845 | precAG288Y1 | ERA1-1 | *recA* | - |
| ERG204S | P1100-P845 | precAD224A1 | ERA1-1 | *recA* | - |
| ERD224A | P1100-P845 | precAE123A3 | ERA1-1 | *recA* | - |
| ERG288Y | P1100-P845 | precAG204S3 | ERA1-1 | *recA* | - |

Table S4 Summary of the phenotypes observed for RecA variants in *recA*-deficient *E. coli* harboring mutant RecA expression plasmid and isogenic mutant *recA* EHEC strains

| | UV sensitivity[a] | Recombination activity[a] | RecA upregulation[a, b] | | LexA cleavage[a] | UmuD cleavage[a] | Stx2 expression[b] | NO response[b] |
|---|---|---|---|---|---|---|---|---|
| | | | a | b | | | | |
| Wild | - | +++++ | + | + | + | + | + | + |
| G22Y | ++ | + | + | - | - | + | + | - |
| G24Y | - | + | + | + | + | + | + | + |
| E123A | ++++++ | - | - | - | - | | - | - |
| G204S | ++ | ++++ | - | - | - | - | - | - |
| D224A | ++++ | - | - | - | - | | - | - |
| G288Y | - | + | + | + | + | | + | + |

[a]These results were reported from Adikesavan et al using *recA*-deficient *E. coli* strains harboring mutant RecA expression plasmid (Adikesavan et al., 2011). UV sensitivity and recombination activity were estimated by UV survival assay and P-1 transduction assay, respectively (Adikesavan et al., 2011). RecA upregulation, LexA cleavage induction and UmuD cleavage induction were analyzed by Western blotting (Adikesavan et al., 2011). The cultures of RecA-wild type or mutant *E. coli* strains were added nalidixic acid (100 μg/ml) and then incubated for 60 min at 37℃ (Adikesavan et al., 2011).

[b]These results were indicated in this study using isogenic mutant *recA* EHEC strains (ERG22Y, ERG24Y, ERE123A, ERG204S, ERD224A and ERG288Y). RecA upregulation and Stx2 production were analyzed by Western blotting. The cultures of wild-typeEHEC or mutant EHEC strains were treated with or without DETA-NONOate (200 μM) and then incubated for 18 h at 37℃ under anaerobic conditions.