Supplementary Figures and Tables

Table S1: RT-qPCR oligonucleotides used in this study

Primer	Forward (5'-3')	Reverse (5'-3')
16S[1]	GCGGTTTGTTAAGTCAGATGTGAAA	GACTCAAGCTTGCCAGTATCAGAT
aatA	CGTCAGCCCCAAAGATAATGAA	TGAGCTTGAGGAGGCGAATA
astA	CCTGGGCTTCCTGGGATAAAA	CACCGGGCAAAAGGCATTTA
bglA	AAGTCGGCTGTATGCTGGCG	CGAACATCACATCGTCCGGGT
cexE	GGCGAGATTGGCGGATATAGCTG	CCTTTAGCAACTGTCTCATTACACGCG
clyA	CGTGTTAGACAGGGTGGTAA	GGGCGTAGTTGAAGGAAAAC
cfaD	TGATTGTAGAGGCATGTCAA	CCAACAAGGTTTTATTCACCT
cfaA	CGGTACAACTGAAGAATATGAAGTTGA	AAGGATAACTTTCTGAGGAGTGACTACA
cfaB	GTTCAAATGCCTATCAGTGTGTCAT	GCAGCAGCTTCAAATTCTTTGG
cfaC	CAATTTGGACGAATGGATCGT	CCTGTCCGTATACCATCAATATCG
cfaE	CTTCTCTGGAAACAAACTGG	CGGAACACTGATTTCTGG
cooA[2]	GTTGACCCGACTGTTGACCT	ACAACGGTGTTGATGGTGTG
cstA[2]	GGCCCACTCTAACCAAAGAAC	AGCCCAAGTTGCATCCAG
iscR	GTACCGTTGGCTGATATTTCCG	CAGACCATTTTTACGCAGACGG
fur	CAGTGCGGAAGATTTATACA	GGTTCAGTACGCGATATACC
fepA	GCGAGCGCGATACCCGTG	TGCCGGACCACGCAGAACTT
fyuA	TGACGGCGACATGATTAACC	AGACGCAGTTTCACATTCCC
gapA	CCTGGCTCCGCTGGCTAAAG	GCGTGAACGGTGGTCATCAGAC
hns	GAAGAAGAAAGCGCGGCTGC	GTCAGCGATCAGCATTTCGCG
ryhB	GCGGAGAACCTGAAAGCACGA	AAAAGCCAGCACCCGGC
ompW	ATGAAGCAGGCGAATTTT	ACCCAGACTTCCTAACGTAC
cgsA	GGCCCAAATTCTGAGCTGAA	AGTCAGAGTTACGGGCATCA
csgE	GATGGGGAAGCTGGATCACAA	CGGAGTCTCTTTTCAACGGGAA
leoA	GTGCAAGCTGCGTCTGAATA	TGTTACTGAGCTGGCAGAGAA
eltB	GATGGCAGGCAAAAG	CGACCTGAAATGTTGC
etpB	GCCCCGGTAAGAAGA	CAGCCCCACATACCC
rplD	ACCTGTTCCTGGCTGCGC	CCGGGTCGATACCAGTTGCA
rpoA	ACCCACGACGGTGATGTTGA	CTCATCGGTCAGGTGGCAGA
sta1 (STp)	CCCCTCTTTTAGTCAGTCAA	CAACAAAGTTCACAGCAGTAA
sta3 (STh)	CTAAACCAGCAGGGTCTT	TGCTTTCAGGACTACTTTCA
tia	GGCGAGATAAGTGATATTTCT	TCCATGCGAAGTTGTTAT
tufB	GGGGCACGCCGACTATGTTA	AACTACCAGGATCGCGCCGT

[1]Oligonucleotide sequences previously described in [1] or [2] [2].

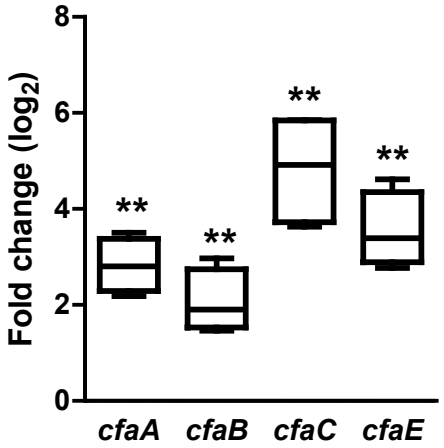

Figure S1. Effect of iron starvation on expression of genes in the CFA/I operon. Gene expression analysis of ETEC H10407 was performed following growth to late-log phase (5 hr) in CFA media alone or treated with 50 μM Deferoxamine. Data are represented as the fold change ratio of expression in treated vs untreated cultures, calculated from relative expression values using the $2^{-\Delta\Delta Ct}$ method and normalized against the 16S housekeeping gene. Values are $\log_2$ transformed. The median is indicated as a band within the box plot. Whiskers represent minimum and maximum values. Statistical analyses were performed using the one sample t-test, **$p<0.01$.

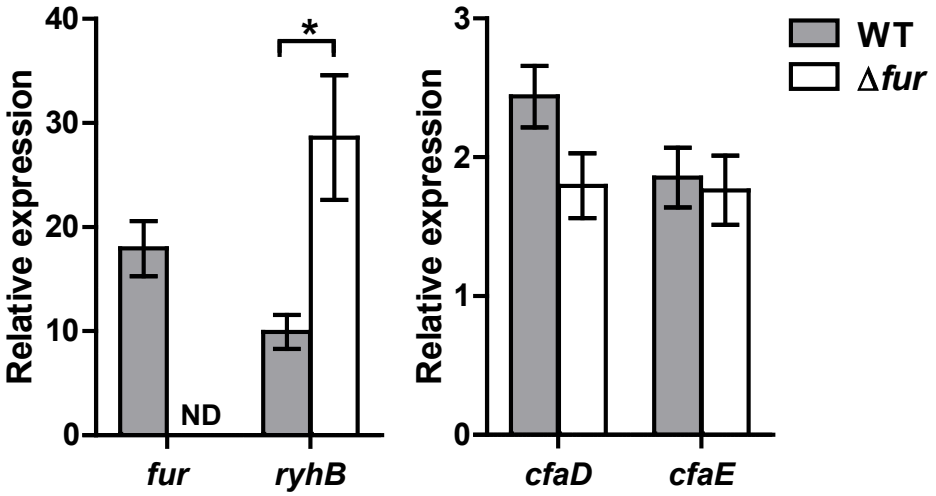

Figure S2. *fur, ryhB*, *cfaD* and *cfaE* gene expression in the H10407 wild-type or Δ*fur* strain in iron-replete media. Gene expression analysis of ETEC H10407 wild-type (WT) or Δ*fur* was performed following growth to early stationary phase (5 hr) in CFA media. Data are represented as relative expression calculated using the $2^{-\Delta\Delta Ct}$ method and normalized against a reference condition. *fur* transcripts were not detected (ND) in the Δ*fur*. Means of at least three independent replicates are shown with error bars representing the SEM. Statistical analyses were performed using the Student t-test, * $p<0.05$.

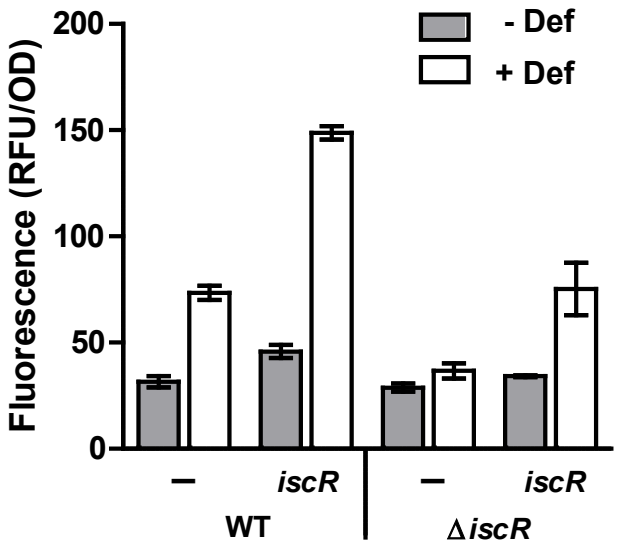

Figure S3. *cfaA* promoter activity with IscR overexpression. The *E. coli* BW25113 wild-type (WT) and isogenic Δ*iscR* mutant strain containing the pPROBE-*cfaA* transcriptional fusion were cultivated in CFA media in presence or absence of 50 µM Deferoxamine (Def). GFP reporter fluorescence was measured following overnight growth in presence of the empty pCA24N vector (-) or in presence of pCA24N-*iscR* (*iscR*). Cultures were performed in presence of 50 µM IPTG to induce *iscR* expression. GFP fluorescence was normalized against bacterial density at 600 nm and expressed as relative fluorescent units (RFU). Means of at least three independent replicates are shown with error bars representing the SEM.

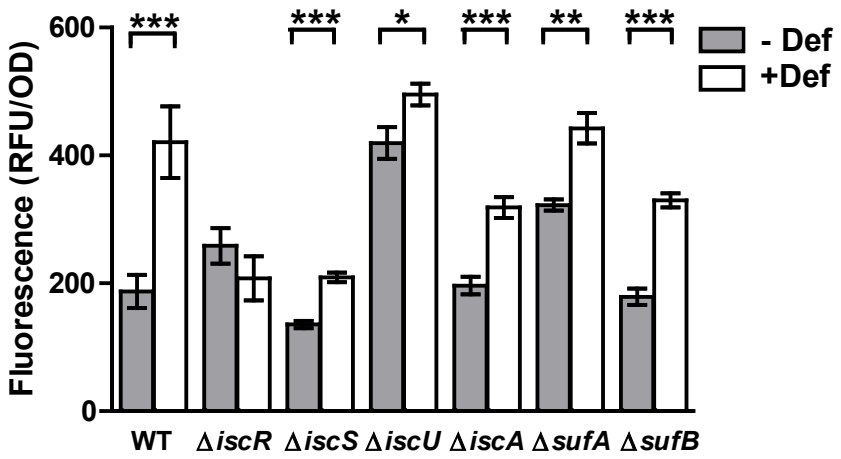

Figure S4: *cfaA* promoter activity in *E. coli* deletion mutants associated with Fe-S cluster biogenesis. *E. coli* BW25113 wild-type and mutant strains containing the pPROBE-*cfaA* transcriptional fusion were cultivated in CFA media in presence or absence of 50 µM Deferoxamine (Def). GFP reporter fluorescence was measured following overnight growth in the presence of pBAD-*cfaD*. Cultures were performed in presence of 0.66 µM L-arabinose for CfaD induction. GFP fluorescence was normalized against bacterial density at 600 nm and expressed as relative fluorescent units (RFU). Means of at least three independent replicates are shown with error bars representing the SEM. Statistical analyses were performed using the Student t-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

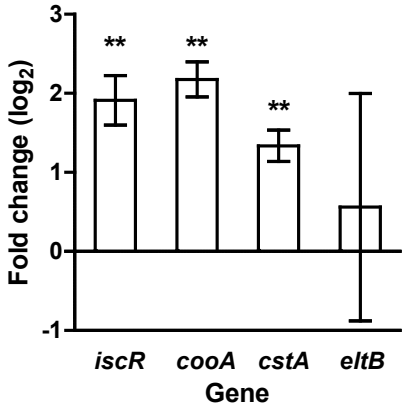

Figure S5: Effect of iron starvation on gene expression in ETEC E24377A. *iscR* (IscR), *cooA* (CS1), *cstA* (CS3), and *eltB* (LT) gene expression was evaluated in stationary phase in CFA media alone or treated with 50 μM Deferoxamine. Data are represented as the fold change ratio of expression in treated vs untreated cultures, calculated from relative expression values using the $2^{-\Delta\Delta Ct}$ method. Values are $\log_2$ transformed. At least three independent replicates were performed with error bars representing the SEM. Statistical analyses were performed using the one sample t-test, **$p<0.01$.

References

1. **Moreno AC, Ferreira LG, Martinez MB.** 2009. Enteroinvasive *Escherichia coli* vs. *Shigella flexneri*: how different patterns of gene expression affect virulence. FEMS microbiology letters **301:**156-163.
2. **Sahl JW, Rasko DA.** 2012. Analysis of Global Transcriptional Profiles of Enterotoxigenic *Escherichia coli* Isolate E24377A. Infection and immunity **80:**1232-1242.