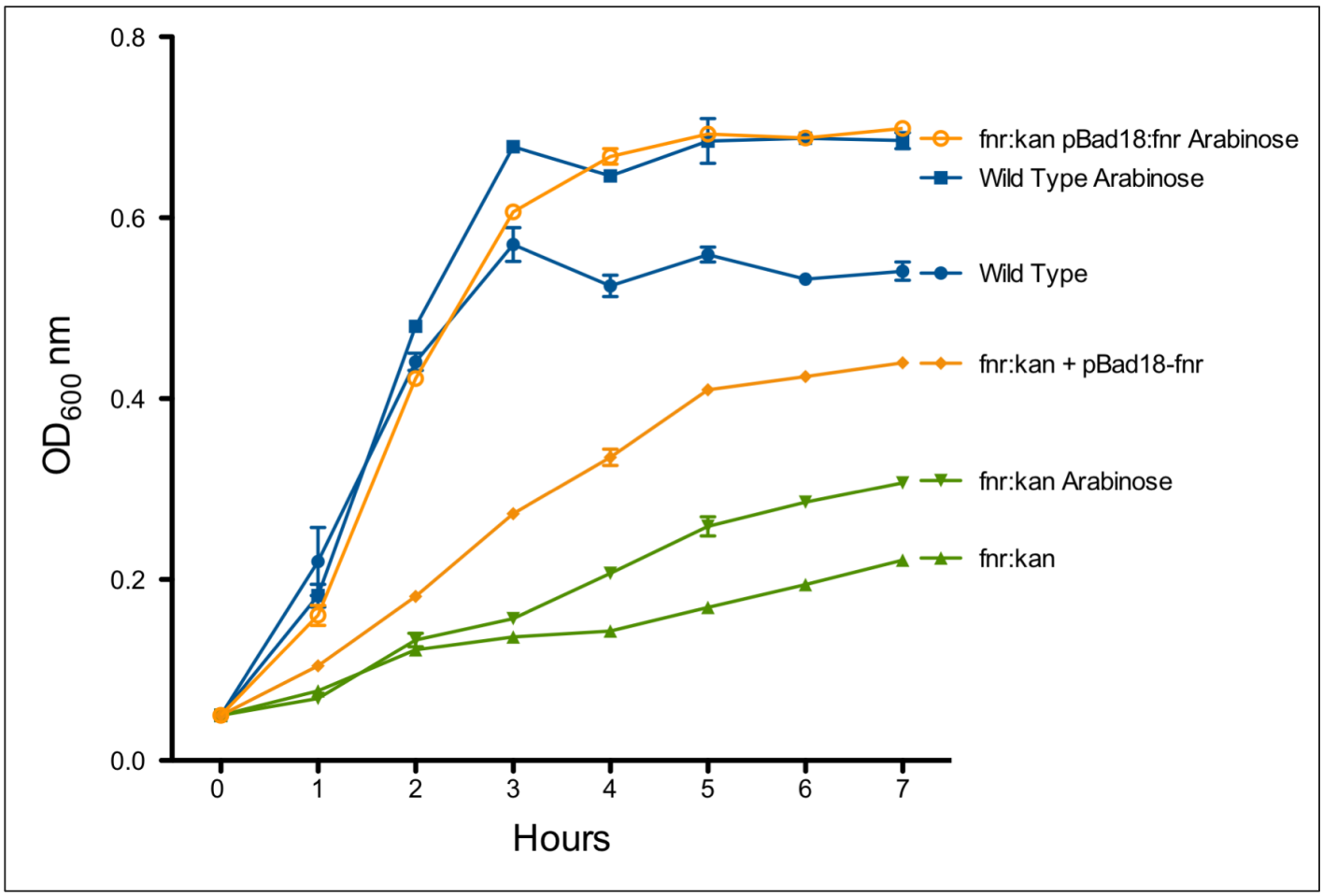
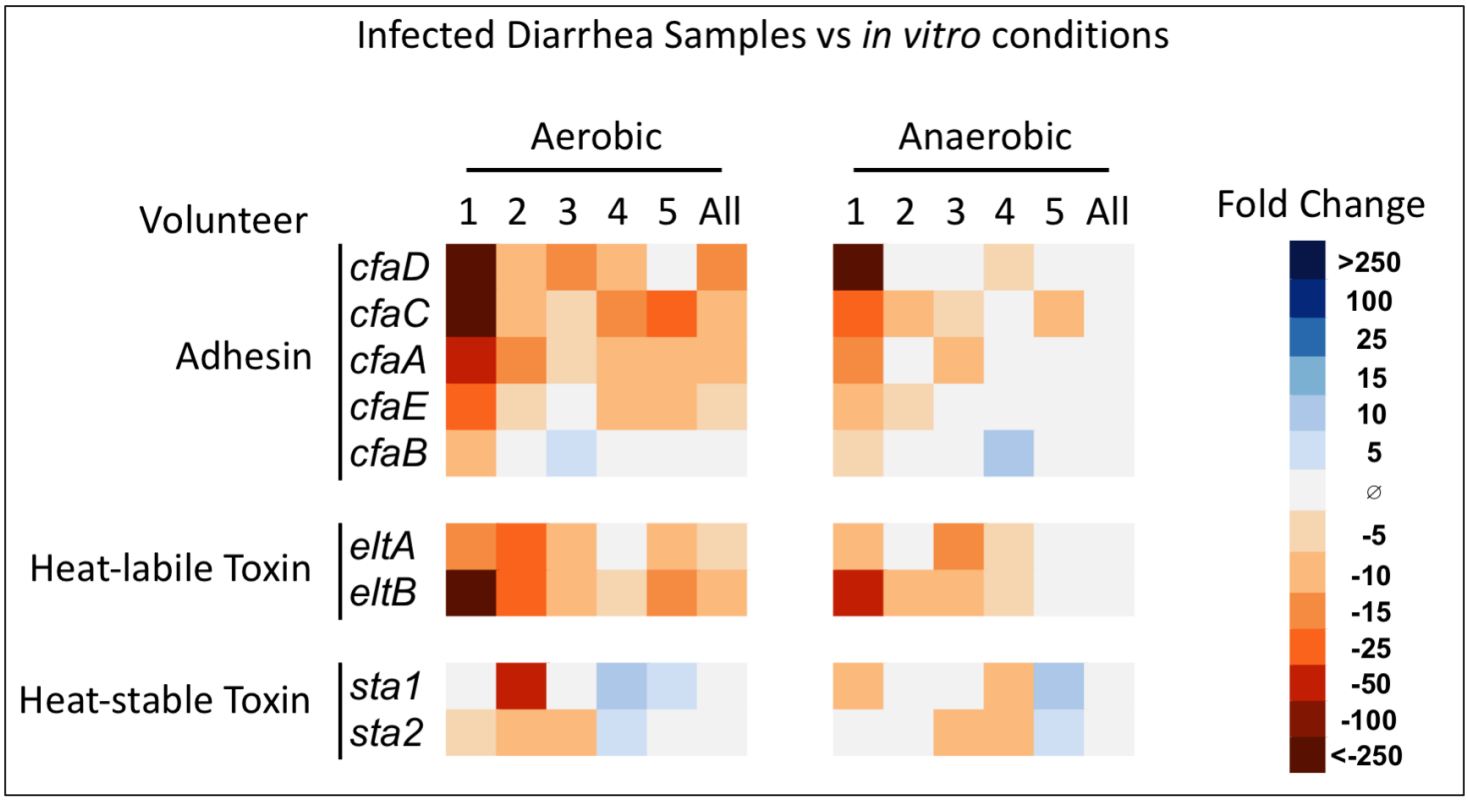
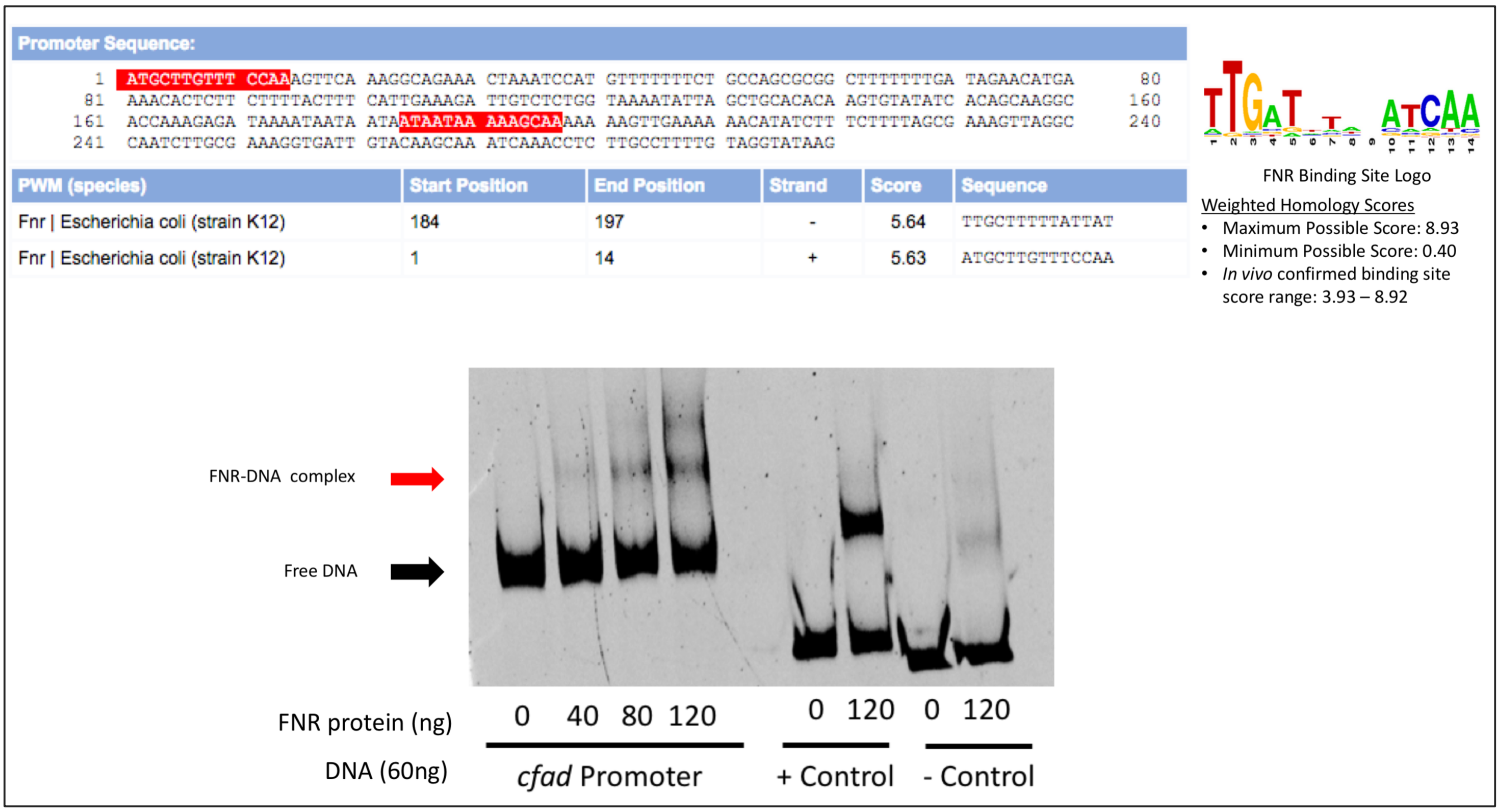
Supplemental Figures (Crofts and co-workers)



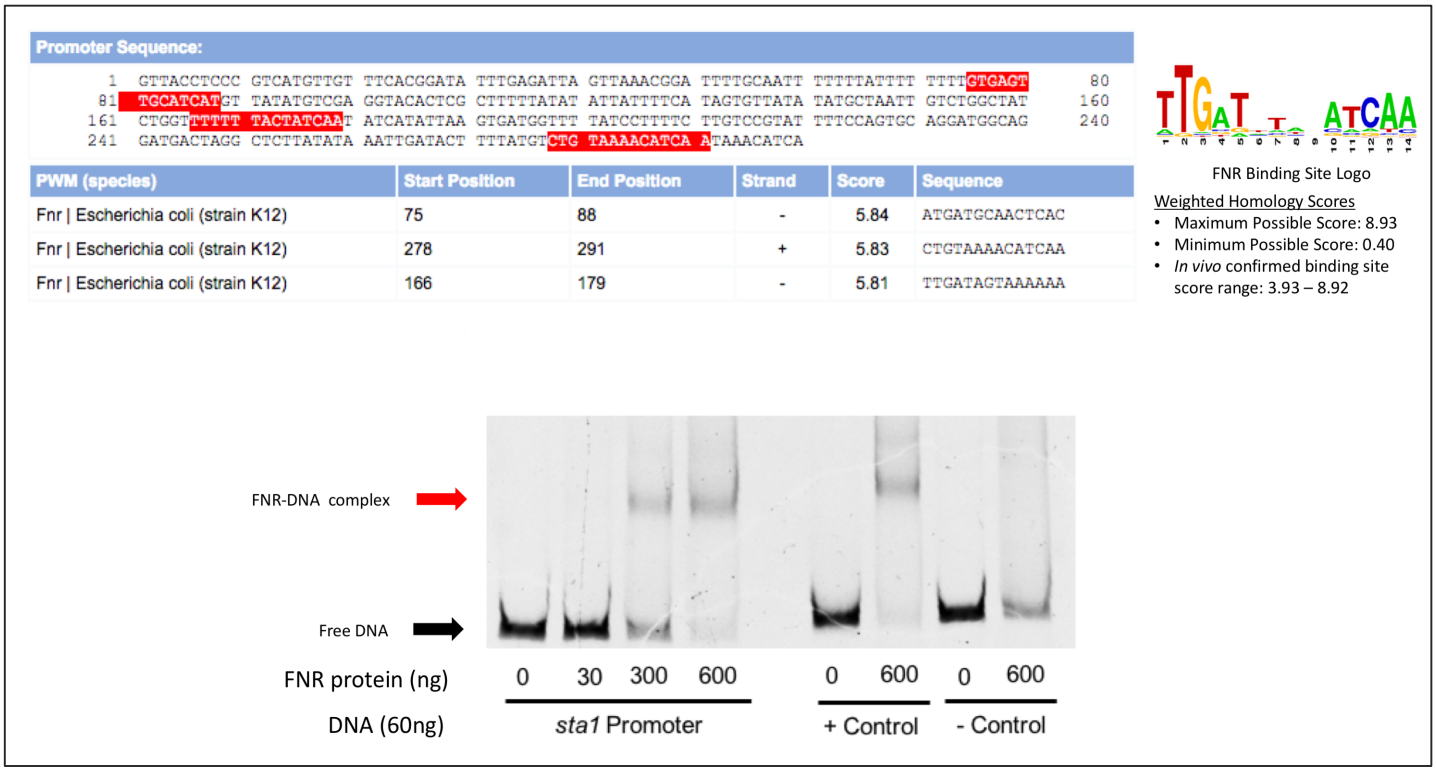
Supplemental Figure 1. Growth curve of ETEC H10407 in anaerobic conditions. All growths represent 3 biological replicates grown in 5 mL CFA broth at 37°C and error bars represents standard error of the mean. Arabinose (0.2%) was added when indicated to induce expression of *fnr* in the pBad18-*fnr* complementation plasmid.



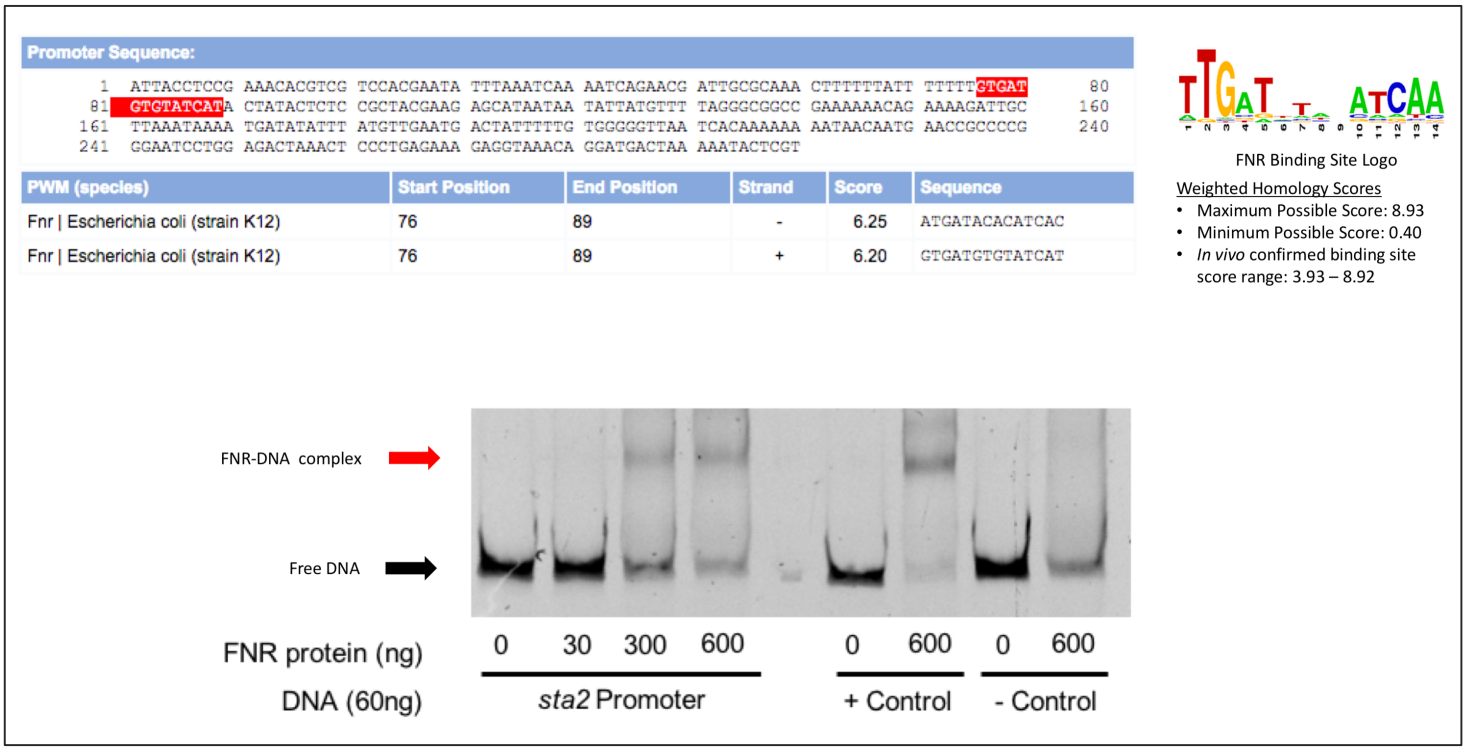
Supplementary Figure 2. Virulence gene expression per volunteer sample. The transcriptome of each volunteer sample was compared to *in vitro* growth in CFA broth under either aerobic or anaerobic atmospheres (3 biological replicates per *in vitro* condition). Classical virulence factor expression is noted per volunteer sample (numbered 1-5) and together as an *in vivo* group representative of all five samples ("All"). Significant fold change was considered > |3| with a false discovery rate correct p value of <0.05 as outline in the methods.



Supplementary Figure 3. *In vitro* binding of FNR to the *cfaD* promoter. **a**. Predicted FNR binding sites in the promoter region of *cfaD* using the online software Prodoric Virtual Footprint Promoter Analysis Version 3.0 online tool to predict *E. coli* K12 FNR binding sites. Binding sites are highlighted in red, and a score indicating how closely the binding sites match the FNR binding site consensus logo are given. A perfect match to the consensus sequence scores a 8.93, and *in vivo* confirmed binding sites have a score range between 3.93 and 8.92 as determined by Prodoric. **b**. Electrophoretic Mobility Shift Assay (EMSA). Purified oxygen stable FNR variant $(FNRD154A)_2$-His6 was mixed at the indicated amounts with PCR amplified *cfaD* promoter DNA (500 base pairs long starting at the -1 position) to test for FNR's ability to bind to promoter DNA. A 500bp region was used to be sure the promoter for *cfaD* was correctly PCR amplified instead of the similar promoter region for the *cfaD2* homolog found elsewhere. FNR-DNA complexes have hindered movement in a 6% polyacrylamide DNA retardation gel which can be seen as a shift in the mobility of the DNA. $(FNRD154A)_2$-His_6 efficiently bound to the *cfaD* promoter and a previously characterized positive control promoter (*ydfZ* gene promoter), but not to a negative control random sequence DNA probe without FNR binding sites.



Supplementary Figure 4. *In vitro* binding of FNR to the *sta1* promoter. **a**. Predicted FNR binding sites in the promoter region of *sta1* using the online software Prodoric Virtual Footprint Promoter Analysis Version 3.0 online tool to predict *E. coli* K12 FNR binding sites. Binding sites are highlighted in red, and a score indicating how closely the binding sites match the FNR binding site consensus logo are given. A perfect match to the consensus sequence scores a 8.93, and *in vivo* confirmed binding sites have a score range between 3.93 and 8.92 as determined by Prodoric. **b**. Electrophoretic Mobility Shift Assay (EMSA). Purified oxygen stable FNR variant $(FNRD154A)_2$-His6 was mixed at the indicated amounts with PCR amplified *sta1* promoter DNA (300 base pairs long starting at the -1 position) to test for FNR's ability to bind to promoter DNA. FNR-DNA complexes have hindered movement in a 6% polyacrylamide DNA retardation gel which can be seen as a shift in the mobility of the DNA. $(FNRD154A)_2$-His_6 efficiently bound to the *sta1* promoter and a previously characterized positive control promoter (*ydfZ* gene promoter), but to not the *sta2* coding sequence used as a negative control (300bp).



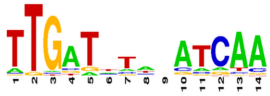
Supplementary Figure 5. *In vitro* binding of FNR to the *sta2* promoter. **a**. Predicted FNR binding sites in the promoter region of *sta2* using the online software Prodoric Virtual Footprint Promoter Analysis Version 3.0 online tool to predict *E. coli* K12 FNR binding sites. Binding sites are highlighted in red, and a score indicating how closely the binding sites match the FNR binding site consensus logo are given. A perfect match to the consensus sequence scores a 8.93, and *in vivo* confirmed binding sites have a score range between 3.93 and 8.92 as determined by Prodoric. **b**. Electrophoretic Mobility Shift Assay (EMSA). Purified oxygen stable FNR variant $(FNRD154A)_2$-His_6 was mixed at the indicated amounts with PCR amplified *sta2* promoter DNA (300 base pairs long starting at the -1 position) to test for FNR's ability to bind to promoter DNA. FNR-DNA complexes have hindered movement in a 6% polyacrylamide DNA retardation gel which can be seen as a shift in the mobility of the DNA. $(FNRD154A)_2$-His_6 efficiently bound to the *sta2* promoter and a previously characterized positive control promoter (*ydfZ* gene promoter), but to not the *sta2* coding sequence used as a negative control (300bp).

a

Promoter Sequence:

```
     1  CGAGGAAAAC TTATATATCA TACAAGAAGA CAATCCGGAA AAAGATAACG CCACTTGTTT TGTTTAACCT ATATGCAACA      80
    81  TGATGTCAAT CATGTTTTTT ATAAAACATC CATGCGAGGA TAAAATGTGT AGTGAAAGCG ATAAAGAAAG AGTGCATCGT     160
   161  TTTCATGCCG GAGACGAATG TGAGAAAGGT GTACATTGAA TCATGGCAAC CTGATTATTT TTATTAGTCG CGACTTGAAC     240
   241  CACGGCAGCT TGGCGGCTGC AAATCGGGGG GTGGCAGAAT ATGAGCTTTC AGAGTAACCA
```

PWM (species)	Start Position	End Position	Strand	Score	Sequence
Fnr | Escherichia coli (strain K12)	80	93	+	6.74	ATGATGTCAATCAT
Fnr | Escherichia coli (strain K12)	80	93	-	6.61	ATGATTGACATCAT
Fnr | Escherichia coli (strain K12)	196	209	-	6.55	TTGCCATGATTCAA

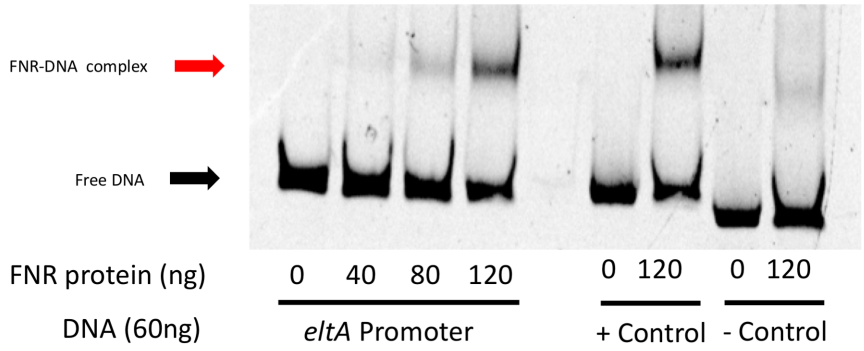


FNR Binding Site Logo

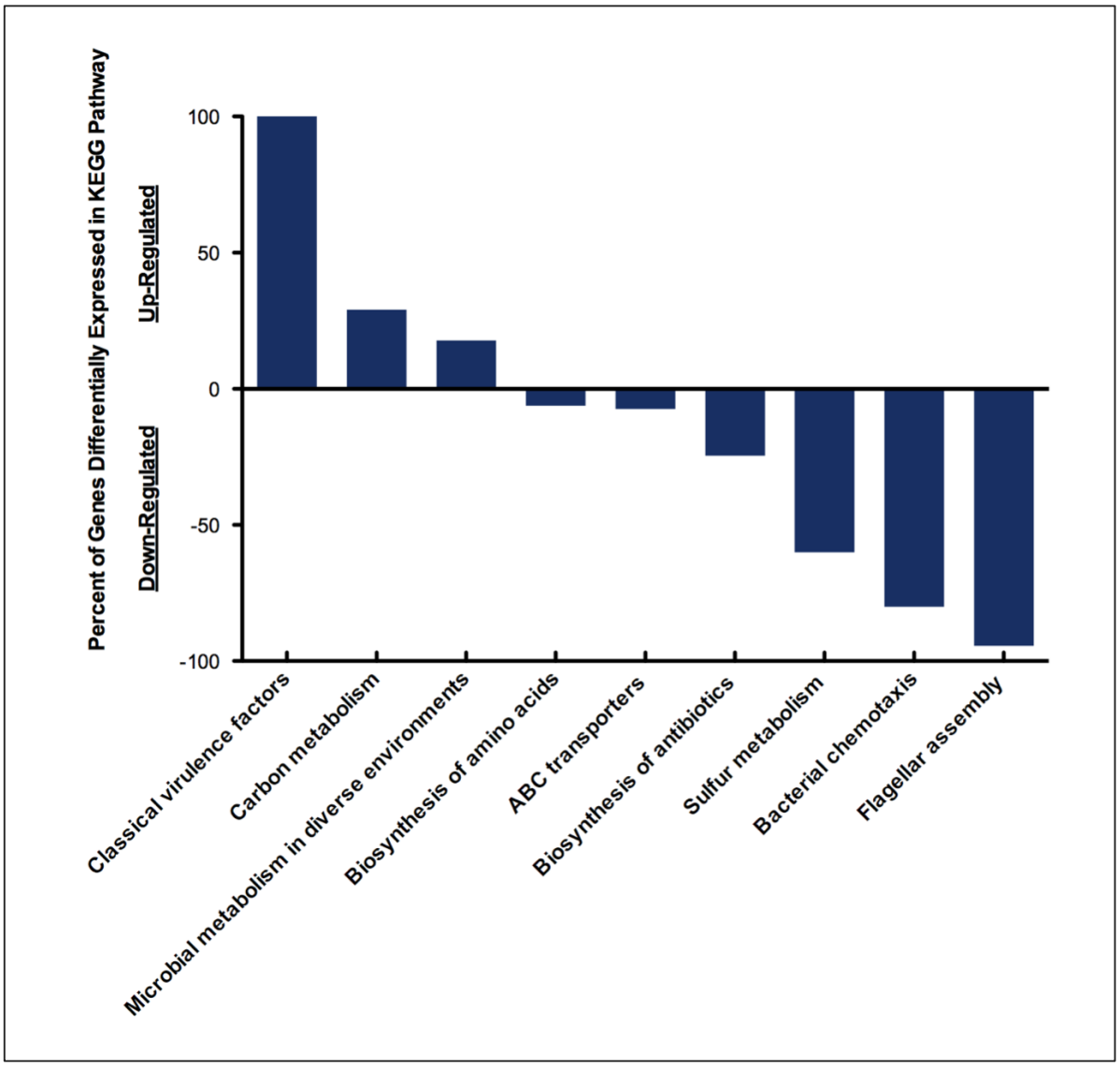
Weighted Homology Scores

- Maximum Possible Score: 8.93
- Minimum Possible Score: 0.40
- *In vivo* confirmed binding site score range: 3.93 – 8.92

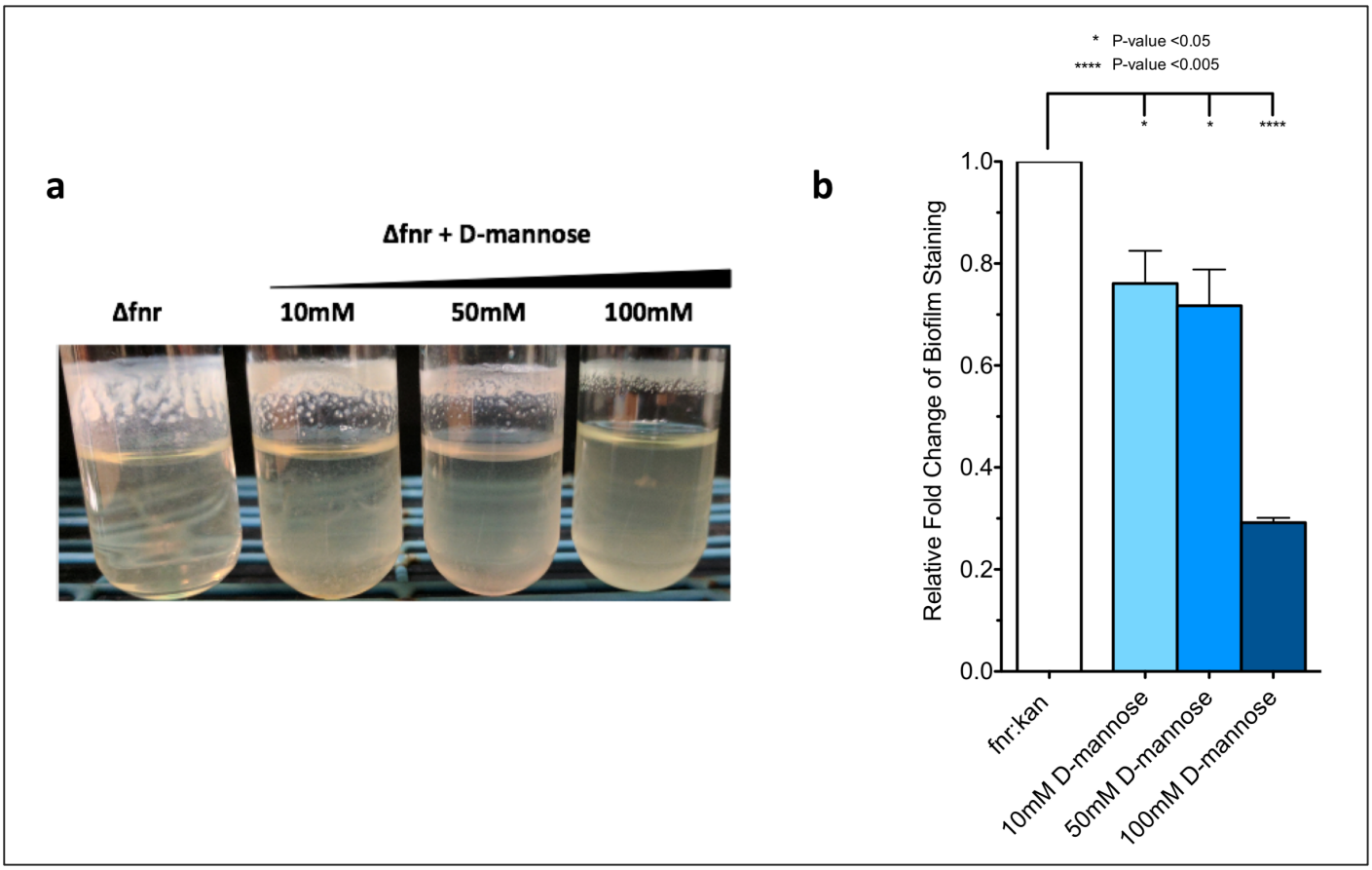
b



Supplementary Figure 6. *In vitro* binding of FNR to the *eltA* promoter. **a**. Predicted FNR binding sites in the promoter region of *eltA* using the online software Prodoric Virtual Footprint Promoter Analysis Version 3.0 online tool to predict *E. coli* K12 FNR binding sites. Binding sites are highlighted in red, and a score indicating how closely the binding sites match the FNR binding site consensus logo are given. A perfect match to the consensus sequence scores a 8.93, and *in vivo* confirmed binding sites have a score range between 3.93 and 8.92 as determined by Prodoric. **b**. Electrophoretic Mobility Shift Assay. Purified oxygen stable FNR variant $(FNRD154A)_2$-His6 was mixed at the indicated amounts with PCR amplified *eltA* promoter DNA (300 base pairs long starting at the -1 position) to test for FNR's ability to bind to promoter DNA. FNR-DNA complexes have hindered movement in a 6% polyacrylamide DNA retardation gel which can be seen as a shift in the mobility of the DNA. $(FNRD154A)_2$-His_6 efficiently bound to the *eltA* promoter and a previously characterized positive control promoter (*ydfZ* gene promoter), but not to a negative control random sequence DNA probe without FNR binding sites.



Supplementary Figure 7. Kegg orthology pathways statistically significantly represented ($p < 0.05$, hypergeometric test) in differentially regulated genes (fold change >|3|, false discovery rate corrected $p < 0.05$ across 3 biological replicate growths, Supplemental Table 1) in an ETEC FNR deletion strain compared to wildtype ETEC. Classical virulence factors were manually curated in the analysis as they are not annotated as such in the KEGG database.



Supplemental Figure 8. FNR regulated biofilm formation in ETEC. **a**. picture of ETEC biofilm formation of an *fnr*:kan strain grown with increasing amounts of D-mannose to quench activity of the fimbrial adhesin protein *fimH*. FimH has been shown previously to contribute to adhesion in ETEC. Representative of at least 3 independent growths. **b**. Quantification of ETEC biofilm formation exemplified in figure Supplementary Figure 5a using optical density readings of crystal violet stained biofilms as described in the methods section. All growths represent three independent biological replicates. Statistical significance was determined using a one-way ANOVA test with tukey multiple comparisons test. Error bars represent standard error of the mean.