

Supplemental material

Supplemental Table 1. Bacterial Strains. Name, genotype and source of all bacterial strains used in this study. Details of construction of strains can be found in full methods for strains constructed in this work, or in the respective cited reference.

Strain	Genotype	Source or Reference
86-24	Stx+ EHEC strain serotype O157:H7	¹
ARP01	86-24 <i>fusK</i> isogenic mutant	This work
ARP02	86-24 <i>fusR</i> isogenic mutant	This work
ARP03	pARP10 in TOP10	This work
ARP04	pARP11 in TOP10	This work
ARP05	pARP10 in <i>fusK</i> -	This work
ARP06	pARP11 in <i>fusR</i> -	This work
ARP15	86-24 <i>z0461</i> mutant	This work
ARP09	<i>fusK</i> - complemented pARP12	This work
ARP10	<i>fusR</i> - complemented pARP13	This work
ARP11	86-24 <i>fusK</i> - <i>fucR</i> - double mutant	This work
ARP12	86-24 <i>ler</i> isogenic mutant	This work
BL21	F- <i>ompT hsdSB(rB-, mB-) gal dcm</i> (DE3)	Invitrogen
TOP 10	Host <i>E. coli</i> strain for protein expression from pBADMyHis vector	Invitrogen
DH5alpha	<i>supE44 D(argF-lac)U169</i> <i>(Δ80dlacΔ(Z)M15) deoR hsdR17 recA1</i>	Stratagene

	<i>endA1 gyrA96 thi-1 relA1</i>	
VS138	<i>qseC</i> isogenic mutant	2
MC474	<i>qseB</i> isogenic mutant	3
NR01	<i>qseE</i> isogenic mutant	4
NR02	<i>qseF</i> isogenic mutant	4

Supplemental Table 2. Plasmids. Name, genotype and source of all plasmids used in this study. Details of construction of plasmids can be found in full methods for plasmids constructed in this work, or in the respective cited reference.

Plasmids	Genotype	Source or Reference
pBADMycHis A	C-terminal Myc-His-Tag vector	Invitrogen
pACYC184	Cloning vector	New England biolabs
pKD3	pANTS λ derivative containing FRT-flanked chloramphenicol resistance	⁵
pKD46	λ red recombinase expression plasmid	⁵
pCP20	TS replication and thermal induction of FLP synthesis	⁵
TOPO PCR blunt	Commercial blunt end cloning vector	Invitrogen
pARP10	FusK in pBADMycHisA	This study
pARP11	FusR in pBADMycHisA	This study
pARP12	<i>fusK</i> in pACYC184	This study
pARP13	<i>fusR</i> in pACYC184	This study
pARP05	<i>fusK</i> in TOPO	This study
pARP06	<i>fusR</i> in TOPO	This study
TOPO fusR-up	326 bp upstream region of fusR in TOPO	This study
pVS154	QseB in pBADMycHis	²

pNR02

| QseF in pBADMycHis

4

Supplemental Table 3. Oligonucleotides. Sequence of oligonucleotides synthesized specifically for this study are listed, sequence for oligonucleotides previously reported in other studies are referenced.

	Primers used for mutagenesis and cloning
Z0462lambdaredP1	TTAATGTCGCGCCAATCTTCGTCATTGGGTGATTTTG CTGTTTATTGTGCTAGCCTGGGGAGTGTAGGTTGGAGCTTGCTTC
Z0462lambdaredP2	CGGGATACCTAAGACTTTTTCTGGTTATCCGGCGATT GTTGCAAAAATGTGGGCAAGTCATATGAATATCCTCCTTA
Z0462F	AAGCTTATTCGCGCAATCTTCG Clone <i>fusK</i> in pBADMyc His
Cz0462rev	AAGCTTATTCGGCGATTTGTTGC Clone <i>fusK</i> in pBADMycHis
Z0462for	GTCGACTCGGCTCGACCACCATCTGC Clone <i>fusK</i> in pACYC184
Z0462rev	TCTAGATGAACGCGCGTGCA Clone <i>fusK</i> in pACYC184
Z0463lambdaredP1	ATTAGATAATAAGAGAAGAAAAGTATGATTCGGGTA GTGCTGGTGGATGACCATGTTGTGGTGTAGGCTGGAGCTGCTTC
Z0463lambdaredP2	TCCCCAGGCTAGCACAATAAACAGCAAAAATCACCCAA TGACGAAGATTGCGCGACATTAACATATGAATATCCTCCTTA
z0463f2	AAGCTTCCATTCGGGTAGTGCTGG Clone <i>fusR</i> in pBADMyc His
Z0463r2	AAGCTTAATGCCCCGCCAGCAG Clone <i>fusR</i> in pBADMyc His
Z0463for	GTCGACGTTGATTGCCAGCGCCGCGC pACYC184 cloning
Z0463rev	TCTAGACCGCCTGTTGACCGTTATTG pACYC184 cloning
Z0461lambdaredP1	GCAATCAGAGTAGAGGTAAAGGTCATTTTCATTGTTTTATCCTTC GAGCGTGAGTGGTGGTGTATGCCCGCTGTGTAGGCTGGAGCTGCTTCG
Z0461lambdaredP2	TTCATATAGTCAGCAACATGGAGACAACCATGCACGCGCGTTCAGCG AGAGAAATTAATCAATGCCATATGAATATCCTCCTTAG

AP-FucR-P1	TAA AGG TCA GTA AAG AAA CCA TTC GTC GCG ATC TCA ATG AAT TAT GTG TAG GCT GGA GCT GCT TCG
AP-FucR-P2	GCT ATC GAT CCC TTC ACA AGA AAA AAT AAA CAG ATC GAT TTC CAG CGA TTC ATA TGA ATA TCC TCC TTA
Ler-lred-P1-	ATG CGG AGA TTA TTT ATT ATG AAT ATG GAA AAT AAT TCA CAT ACA ACA AG GTGTAGGCTGGAGCTGCTTCG
Ler-lred-P2	TTC AGT GTC CTT CAC AAG AAA ATC TTC TTT CTT CAT TCC ATT CAA CAG TGC ATA TGA ATA TCC TCC TTA

Oligonucleotides used for Real time PCR

Ler	6
escC	6
escV	6
Eae	6
espA	6
rpoA	6
Z0461-201F1	AACCCGCCGCAGCTTT
Z0461-262R1	TGGCCACAAATGCTGATT
Z0463-343R1	CGGTGCCAGCGGGTATT
Z0463F	GCGCACCGCCTGTACTAACT
afuAF-887F1	TCGCCAAACGCTTTGGTT

afuB-1809F1	AGCCGGCATCGAAATAATGAC
afuB-1870R1	GGTCCGCCCGTTTCGT
afuC-894F1	CCAGACGCAAAATGGTGGTT
afuC-952R1	GCAAATGGTGACGCTGCTT
fucA-269F	GGCGCGCAAGGAATAGAA
fucA-328R	GATCCCCGCTATTCACTACATGA
fucP-1139F	CCAAATACGGTTCGTCCTTCA
fucP-1206R	ACCCATGACCGGAGTGACAA
fucR-260F	CGCTCGCGTGGATTGAA
fucR-326R	GCCAGATACCAGCAAGTTGAACT

Primers used for EMSAs

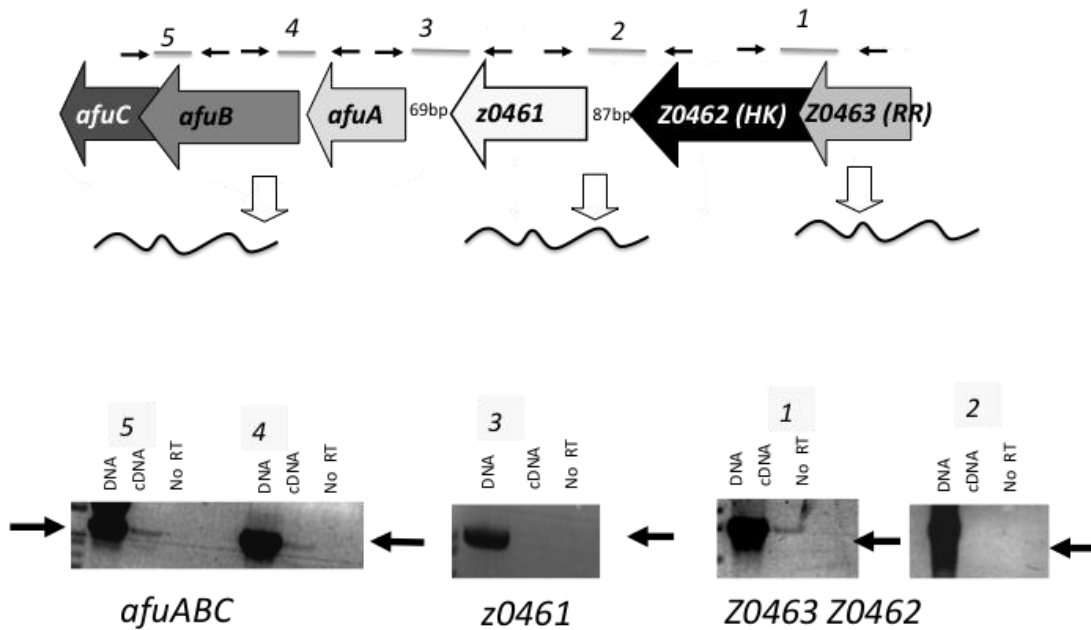
Ler-299FP	5'-ATGCAATGCGATCTATCTATC-3'
Ler-18FPr	5'-GGAAGGACCAATTAATC-3'
Ler+86R	5'-GCTTCCTGCTGTAGAACTGC-3'
Ler-42F	5'-GTGGTTGTTTGATGAAATAGATGTG-3'
Ler-218F	5'-CGCTTAACTAAATGGAAATGC-3'
KanF	5'-CCGGAATTGCCAGCTGGGGCG-3'
KanR	5'-TCTTGTTCAATCATGCGAAACGATCC-3'

Supplemental Table 4. Number of genes with altered expression in Z0462- and Z0463- compared to WT. The GeneChip *E. coli* Genome 2.0 array system of the Affymetrix system was used to compare the gene expression in strain 86-24 to that in *fusK*- and *fusR*- strains. The output from the scanning of the Affymetrix GeneChip® *E. coli* 2.0 were obtained using GCOS v 1.4 according to manufacturer’s instructions. Comparisons were performed using the analysis tools within GCOS v 1.4, by selecting the appropriate array, CHP file for comparison, and baseline values. Custom analysis scripts were written in Perl to complete multiple array analyses. Expression data can be accessed using accession number (GSE34991) at the NCBI GEO database.

	Increased	Decreased	No Change
Z0462-	785	433	8279
Z0463-	660	273	8004

Supplemental Table 5. Metabolic genes in the gene arrays. None of these had their expression changed.

EHEC <i>fusK</i> -		EHEC <i>fusR</i> -			
0.1 no change	0.5 rpiA	0.5 no change	0.069813	rpiA	ribose-5-phosphate isomerase A
0 no change	0.532344 yggF	-0.6 no change	0.916174	yggF	fructose-1,6-bisphosphatase II-like protein
-0.2 no change	0.5 yqgP	-0.5 no change	0.99288	yqgP	putative oxidoreductase
0 no change	0.5 cmtA	-0.8 no change	0.990708	cmtA	predicted fused mannitol-specific PTS enzymes: IIB component/IIC component
-0.9 no change	0.990708 galP	-1.5 no change	0.988955	galP	D-galactose transporter
-0.5 no change	0.5 ebgA	-0.9 no change	0.747149	ebgA	cryptic beta-D-galactosidase, alpha subunit
0.2 no change	0.118009 ebgC	-1 no change	0.952736	ebgC	cryptic beta-D-galactosidase subunit beta
1.8 no change	0.124552 ygjK	1.1 no change	0.5	ygjK	putative glycosyl hydrolase
-2.7 no change	0.900148 exuT	-1.3 no change	0.838962	exuT	hexuronate transporter
0.2 no change	0.5 agaV	-1.7 no change	0.995927	agaV	N-acetylgalactosamine-specific PTS system transporter subunit IIB
-0.7 no change	0.715033 agaW	-1.5 no change	0.990708	agaW	N-acetylgalactosamine-specific PTS system enzyme IIC component
0.2 no change	0.5 agaB	0.1 no change	0.545234	agaB	putative N-acetylgalactosamine-6-phosphate deacetylase
1.6 no change	0.5 agaC	1.4 no change	0.441923	agaB	N-acetylgalactosamine-specific PTS system transporter subunit IIB
2.5 no change	0.177412 agaD	1.2 no change	0.5	agaC	N-acetylgalactosamine-specific PTS system transporter subunit IIC
0.4 no change	0.60871 agaD	-0.6 no change	0.934434	agaD	N-acetylgalactosamine-specific PTS system transporter subunit IID
0.3 no change	0.62112 agaI	-2.2 no change	0.975245	agaI	galactosamine-6-phosphate isomerase
-0.1 no change	0.39129 yraM	-0.6 no change	0.996301	yraM	putative glycosylase
-0.6 no change	0.930187 ptsN	-0.3 no change	0.558077	ptsN	sugar-specific enzyme IIA component of PTS
-1.1 no change	0.5 nanE	-0.6 no change	0.934434	nanE	N-acetylmannosamine-6-phosphate 2-epimerase
1.3 no change	0.016731 nanT	0.3 no change	0.354442	nanT	putative sialic acid transporter
0.9 no change	0.01421 nanR	-0.2 no change	0.5	nanR	N-acetylneuraminate lyase
0.7 no change	0.009292 nanR	0.1 no change	0.177412	nanR	transcriptional regulator NanR
-0.5 no change	0.5 frlB	-1 no change	0.997247	frlB	fructoselysine-6-P-deglycase
0.2 no change	0.044149 frlC	-0.4 no change	0.5	frlC	fructoselysine 3-epimerase
2 no change	0.038415 gntT	1.9 no change	0.054022	gntT	gluconate permease
-0.2 no change	0.757415 malQ	no change		malQ	4-alpha-glucanotransferase
-0.5 no change	0.921063 malP	no change		malP	malto-dextrin phosphorylase
-0.1 no change	0.5 glgC	-1 no change	0.998168	glgC	glucose-1-phosphate adenylyltransferase
0 no change	0.5 glgX	-1.1 no change	0.998349	glgX	glycogen debranching enzyme
-0.4 no change	0.5 glgB	-1.5 no change	0.999899	glgB	glycogen branching enzyme
1.2 no change	0.274048 gntU	0.4 no change	0.493524	gntU	low affinity gluconate transporter
0.7 no change	0.026698 gntU	-0.5 no change	0.805199	gntU	low affinity gluconate transporter
0.6 no change	0.083826 ugpE	-1 no change	0.977068	ugpE	glycerol-3-phosphate transporter membrane protein
1.5 no change	0.008511 ugpA	0.1 no change	0.757415	ugpA	glycerol-3-phosphate transporter permease
0 no change	0.124552 ugpB	-0.8 no change	0.930187	ugpB	glycerol-3-phosphate transporter periplasmic binding protein
0.7 no change	0.153232 dctA	-0.8 no change	0.757415	dctA	C4-dicarboxylate transporter DctA
0.2 no change	0.5 xylB	0.5 no change	0.001486	xylB	xylulokinase
-0.7 no change	0.747149 xylA	-0.5 no change	0.5	xylA	xylose isomerase
-0.7 no change	0.796129 xylF	-1.9 no change	0.981872	xylF	D-xylose transporter subunit XylF
-0.2 no change	0.274048 xylG	-1.1 no change	0.796129	xylG	xylose transporter ATP-binding subunit
0.4 no change	0.088938 xylH	0.2 no change	0.153232	xylH	putative membrane component of xylose transport system
1.7 no change	0.061522 uhpT	-0.3 no change	0.938478	uhpT	sugar phosphate antiporter
-1.2 no change	0.991489 uhpA	-2.2 no change	0.961585	uhpA	DNA-binding transcriptional activator UhpA
-0.5 no change	0.506476 bglB	0.9 no change	0.681091	bglB	6-phospho-beta-glucosidase bglB
-2.8 no change	0.992212 bglF	-5.4 no change	0.986922	bglF	fused beta-glucoside-specific PTS enzymes: IIA component/IIB component/IIC component
-0.3 no change	0.681091 rbsA	-0.9 no change	0.996645	rbsA	D-ribose transporter ATP binding protein
-0.8 no change	0.894337 rbsC	-1.3 no change	0.995927	rbsC	ribose ABC transporter permease protein
0 no change	0.506476 frvB	-1.5 no change	0.945978	frvB	putative PTS transporter components IIBC
0.5 no change	0.008511 frvA	-3.1 no change	0.757415	frvA	putative fructose-like phosphotransferase system subunit EIIA
0.8 no change	0.041201 rhaM	-0.2 no change	0.645558	rhaM	L-rhamnose mutarotase
-1.5 no change	0.852061 rhaD	-1.1 no change	0.814869	rhaD	rhamnulose-1-phosphate aldolase
-1.1 no change	0.822588 rhaA	-1.5 no change	0.545234	rhaA	L-rhamnose isomerase
0.7 no change	0.153232 rhaB	-1.6 no change	0.938478	rhaB	rhamnulokinase
0.1 no change	0.145682 rhaS	0.2 no change	0.274048	rhaS	transcriptional activator RhaS
0.5 no change	0.013078 rhaR	-0.1 no change	0.454766	rhaR	transcriptional activator RhaR
-0.5 no change	0.757415 glpX	-1.2 no change	0.830902	glpX	fructose 1,6-bisphosphatase II
0.7 no change	0.083826 glpK	0.1 no change	0.5	glpK	glycerol kinase
0.3 no change	0.153232 frwC	-1 no change	0.894337	frwC	putative fructose-like permease EIIC subunit 2
-1.3 no change	0.94878 frwB	-1.2 no change	0.5	frwB	putative fructose-like phosphotransferase EIIB subunit 2
0.9 no change	0.065566 xylE	-0.5 no change	0.905721	xylE	D-xylose transporter XylE
-0.7 no change	0.966696 malG	-1.9 no change	0.99997	malG	maltose transporter permease
-0.1 no change	0.875448 ytfT	-1.2 no change	0.999135	ytfT	predicted sugar transporter subunit: membrane component of ABC superfamily
-0.5 no change	0.822588 yjfF	-0.3 no change	0.952736	yjfF	predicted sugar transporter subunit: membrane component of ABC superfamily
0.5 no change	0.274048 fbp	1.2 no change	0.004481	fbp	fructose-1,6-bisphosphatase
0.2 no change	0.019624 nagC	0 no change	0.330589	nagC	transcriptional repressor of N-acetylglucosamine operon
0.2 no change	0.013078 nagE	-0.1 no change	0.5	nagE	N-acetyl glucosamine specific PTS system components IIAABC
-0.4 no change	0.429141 Z2474m	-3.1 no change	0.875448	Z2474m	sugar-binding periplasmic protein
2.4 no change	0.111714 Z2474m	-1.9 no change	0.506476	Z2474m	sugar-binding periplasmic protein
1.6 no change	0.330589 ycjM	-0.5 no change	0.900148	ycjM	glycosidase
-0.1 no change	0.715033 murQ	-1.9 no change	0.973302	murQ	N-acetylmuramic acid-6-phosphate etherase
0.2 no change	0.5 murP	-0.8 no change	0.5	murP	N-acetylmuramic acid phosphotransfer permease



Supplementary Figure 1. Genetic organization of OI-20 depicting the number base pairs (bp) between the different transcriptional units. Operonic structure of OI-20 by RT-PCR. . Numbers 1-5 show location of primer sets used to map junction between genes. Primers (1) flanking *z0462* and *z0463* show no product when no RT is added (No RT) and product when using either the genomic DNA (DNA) control or cDNA (cDNA), suggesting that *z0462* and *z0463* are co-transcribed. Primers (2) flanking *z0462* and *z0461* show no product when no RT is added (No RT) or with cDNA (cDNA), and product when using genomic DNA (DNA), suggesting that *z0462* and *z0461* are in independent transcriptional units. Primers (3) flanking *z0461* and *afuA* show no product when no RT is added (No RT) or with cDNA (cDNA), and product when using genomic DNA (DNA), suggesting that *z0461* and *afuA* are in independent transcriptional units. Primers (4) flanking *afuA* and *afuB* show no product when no RT is added (No RT) and product when using either the genomic DNA (DNA) control or cDNA (cDNA), suggesting that *afuA* and *afuB* are co-transcribed. Primers (5) flanking *afuB* and *afuC*

show no product when no RT is added (No RT) and product when using either the genomic DNA (DNA) control or cDNA (cDNA), suggesting that *afuB* and *afuC* are co-transcribed

Z0462 (HK)

Putative sensor kinase

Search for sequence similarity (BLAST)

- EHEC
- *Citrobacter rodentium*



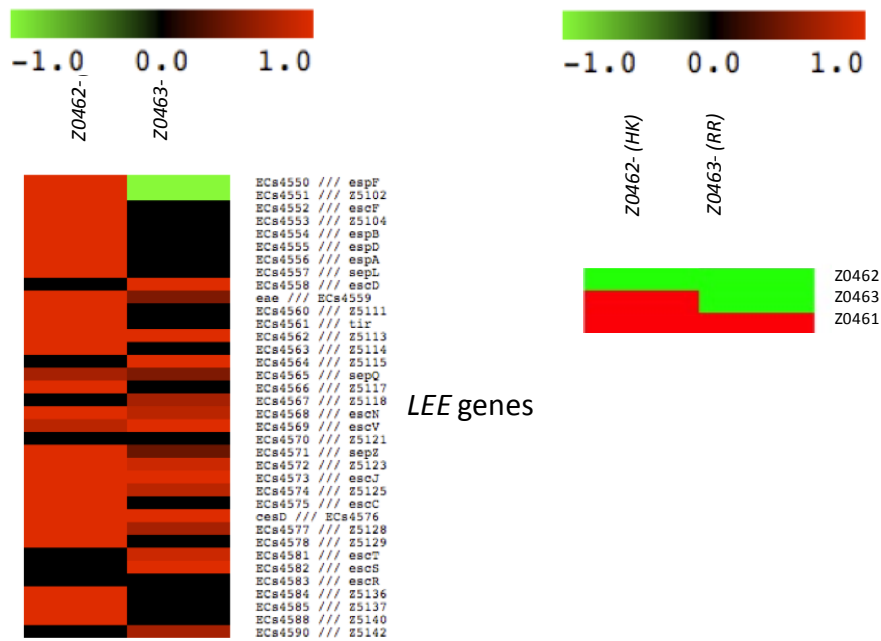
Z0463 (RR)

Putative response regulator

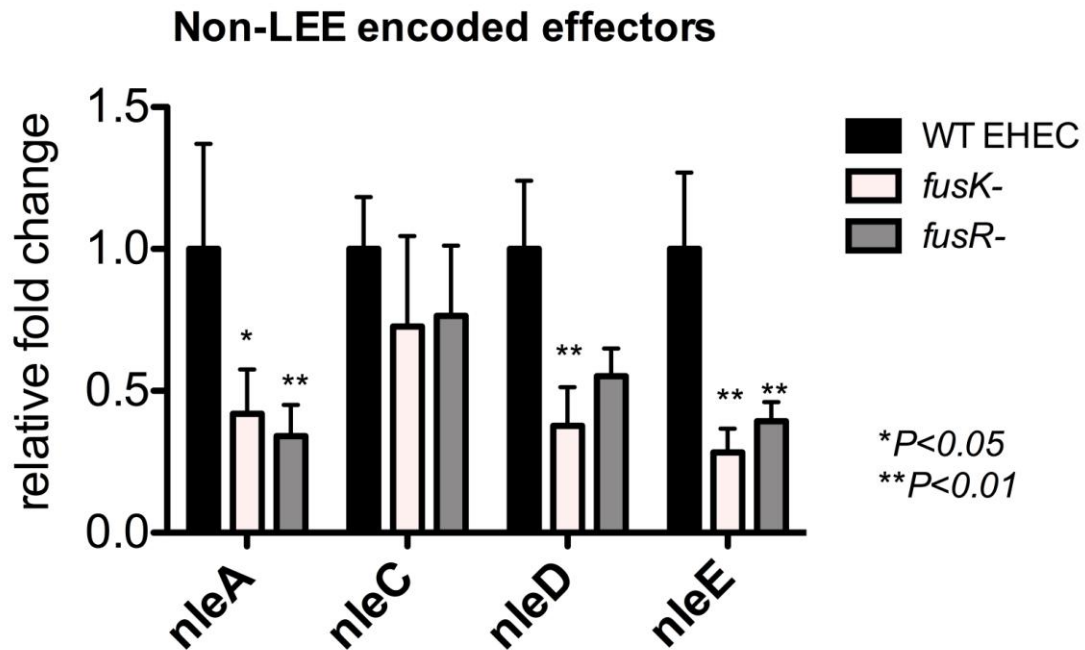
- EHEC
- *Citrobacter rodentium*



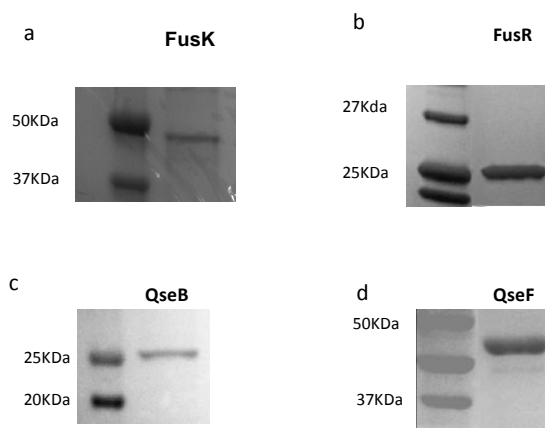
Supplementary Figure 2. Domain organization of Z0462 and Z0463.



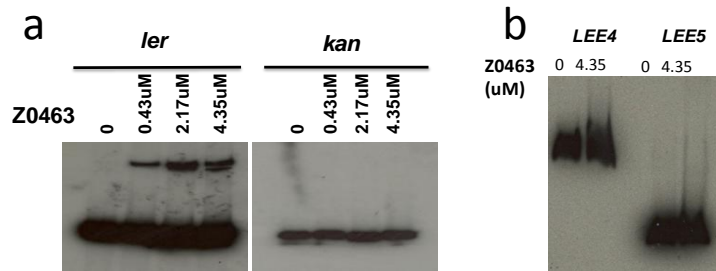
Supplementary Figure 3. Heat map from microarray analysis representing genes with altered expression in the *z0462-* and *z0463-* compared to WT.



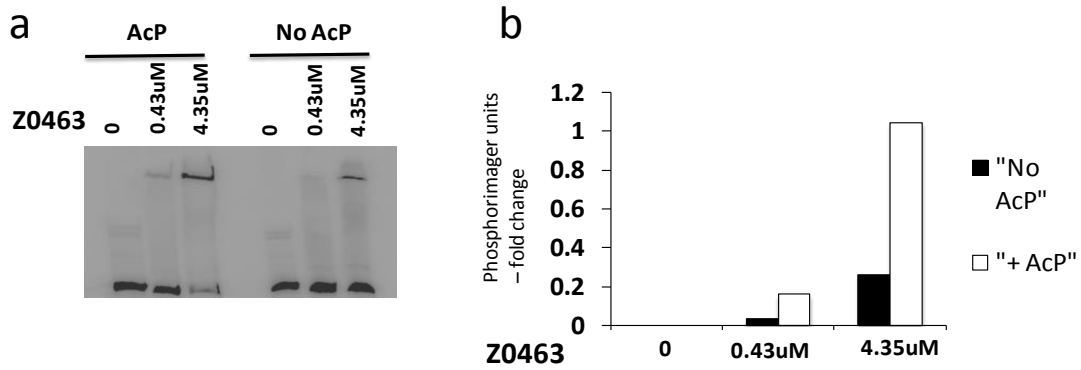
Supplementary Figure 4. qRT-PCR of genes encoding non-LEE effectors (*nle*). RNA was extracted from strains grown in DMEM to an OD_{600} 1.0. ($n=18$ biological samples per strain; asterisk, $P < 0.05$; two asterisks, $P < 0.01$; Student's *t*-test). These data show that expression of *nleA*, *nleD* and *nleE* is decreased in the *fusK* and *fusR* mutants suggesting that FusKR activates expression of these genes. Expression of *nleC* is unaltered in these mutants, suggesting that *nleC* is not regulated by FusKR.



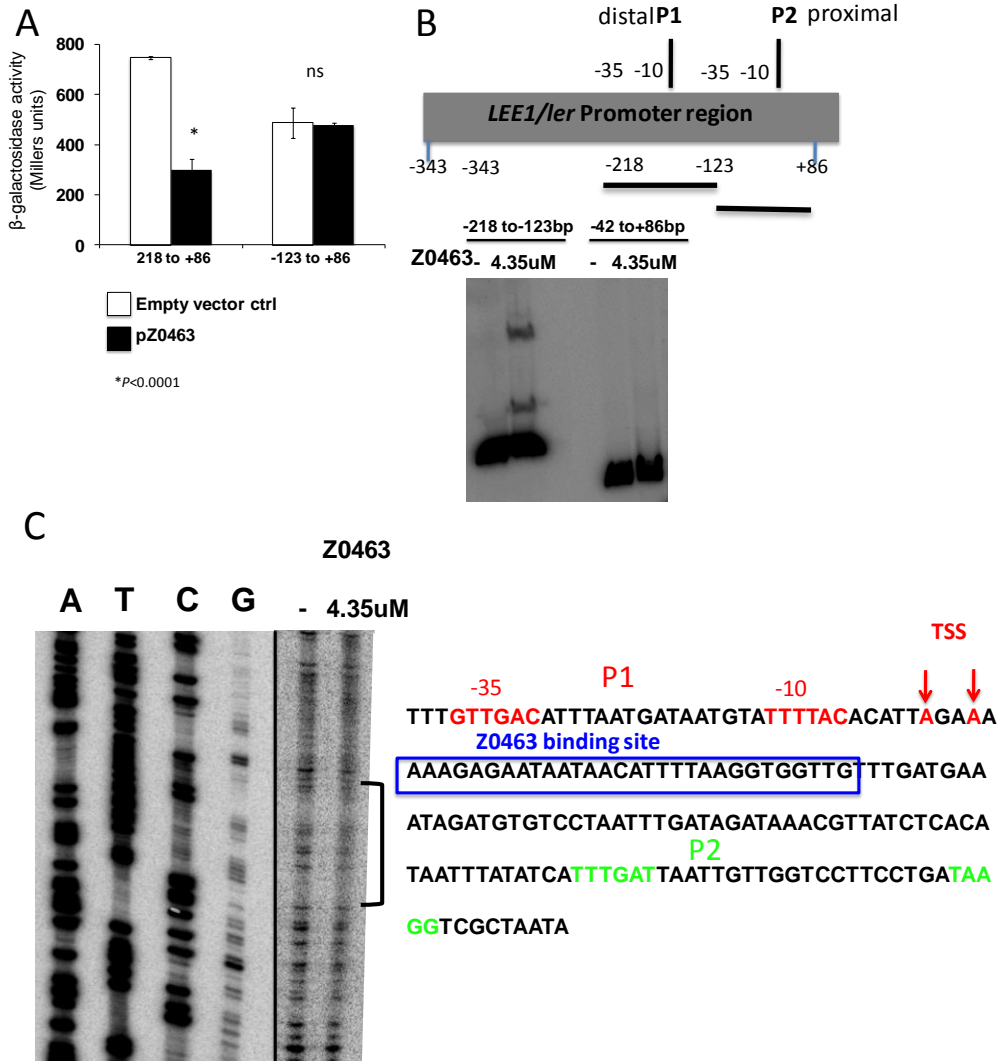
Supplementary Figure 5. Commassie gels depicting the purified proteins used in this study. a, Commassie gel of purified FusK. **b,** Commassie gel of purified FusR. **c,** Commassie gel of purified QseB. **d,** Commassie gel of purified QseF



Supplementary Figure 6. a, EMSAs of *ler* and *kan* (negative control) probes with Z0463. These data show that Z0463 repress transcription of *ler* by directly interacting with its promoter. **b**, EMSAs of *LEE4* and *LEE5* probes with Z0463. These data show that repression of *LEE4* and *LEE5* transcription by Z0463 is indirect, and probably occurs in a cascade fashion through Ler.



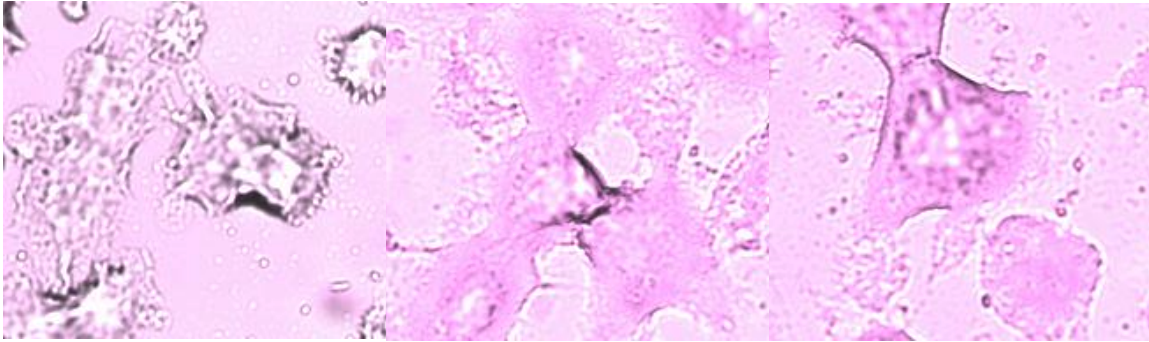
Supplementary Figure 7. a, EMSA of *ler* with Z0463 in the absence and presence of acetyl phosphate (AcP). These data demonstrate that Z0463 binds to the *ler* promoter with higher affinity when phosphorylated. **b**, Quantification of bound probe with and without AcP. Quantification was performed using a Storm phosphorimager.



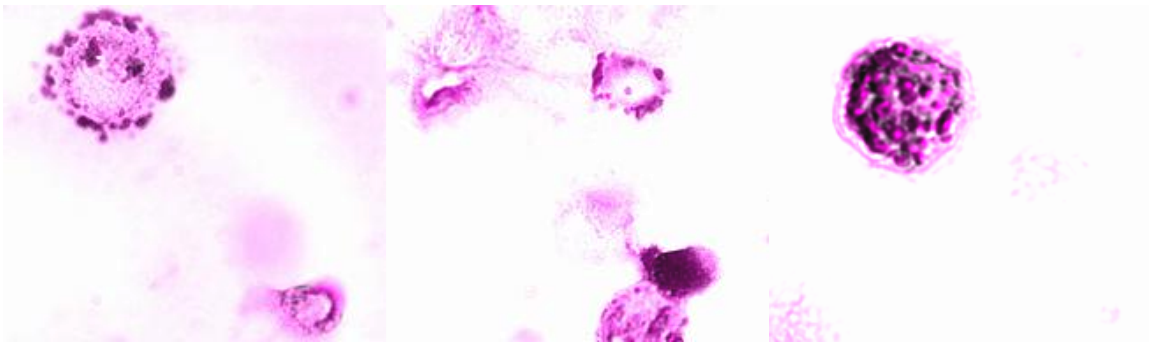
Supplementary Figure 8. Z0463 directly repress *ler* (*LEE1*) transcription. **a**, Nested deletion analyses of the *LEE1* (*ler*) promoter region. Transcriptional fusions of different fragments from the *LEE1* regulatory region (-218 to +86bp and -123 to +86bp) were generated and introduced into the K-12 MC4100 strain genome (does not have the Z0463), and assayed for β -lactamase activity in the presence of empty vector or Z0463. ($n=6$ biological samples per strain; asterisk, $P<0.0001$; ns, $P>0.05$, Student's *t*-test). **b**, EMSA of smaller fragments of the *LEE1* promoter with Z0463 (with acetyl phosphate). **c**, DNA footprinting of the *LEE1* promoter with Z0463. The two *LEE1* promoters are

depicted in red (P1) and green (P2), and the Z0463 binding region is boxed in blue. These data show that Z0463 binds in a region within the P1 and P2 promoters to repress *ler* transcription.

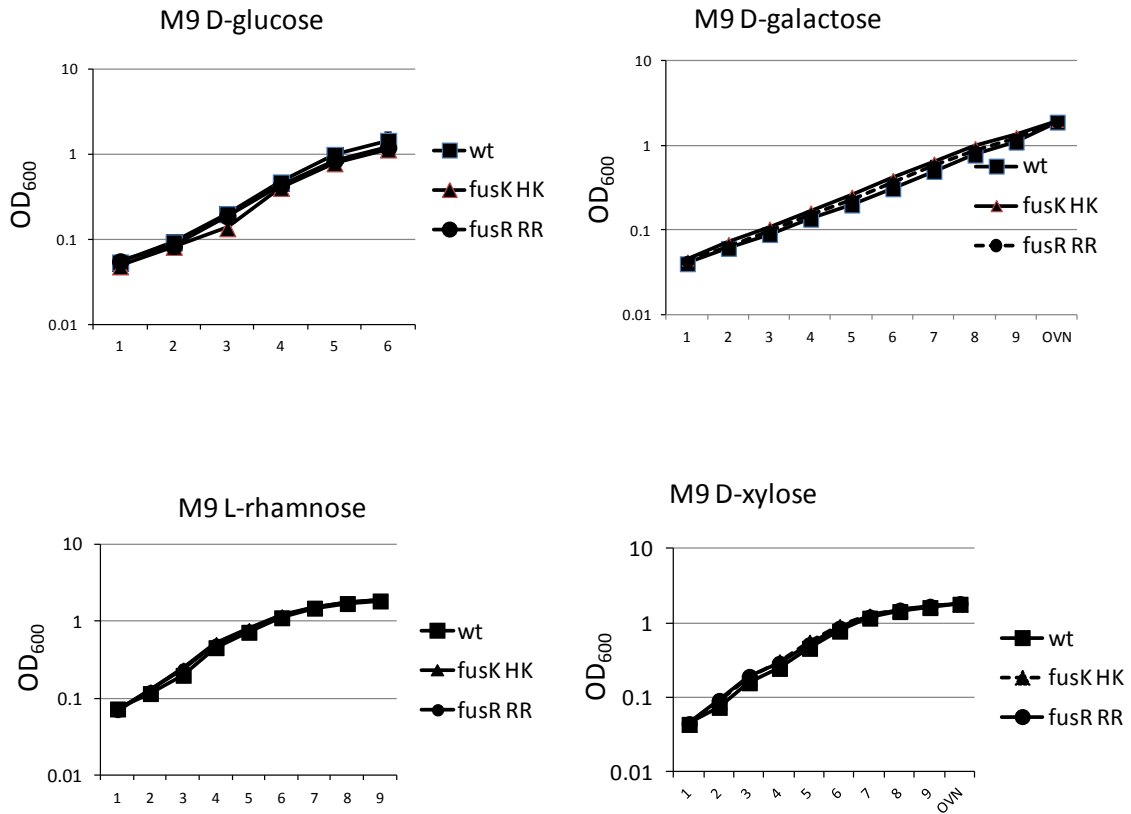
Undifferentiated HT29 cells



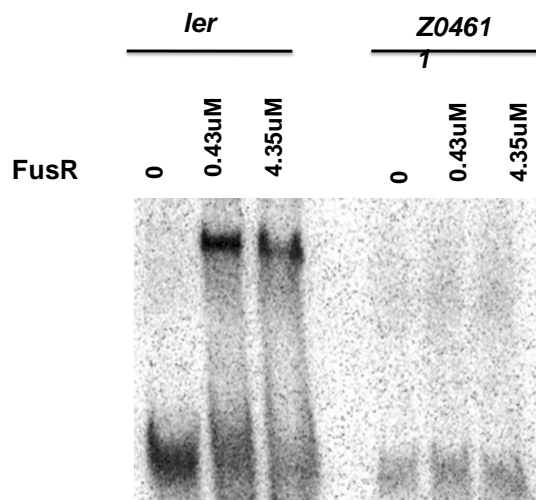
Differentiated HT29 cells



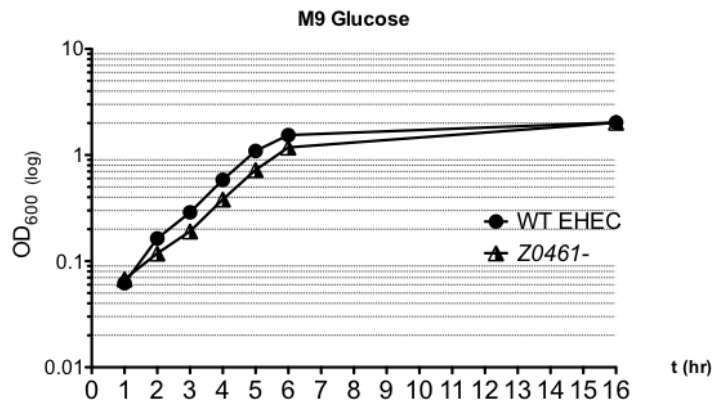
Supplementary Figure 9. Propionic acid Schiff staining (PAS) of undifferentiated (non mucus producing) and differentiated (mucus producing) HT29 cells.



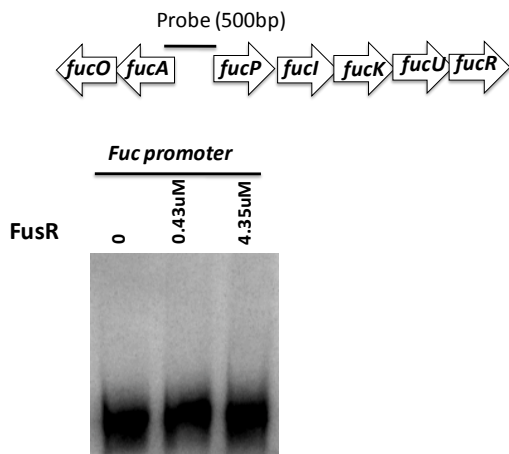
Supplementary Figure 10. Growth curves in M9 minimal media with different carbon sources. ($n=6$ biological samples; significance between generation times calculated through Anova). No significant differences in generation times were observed.



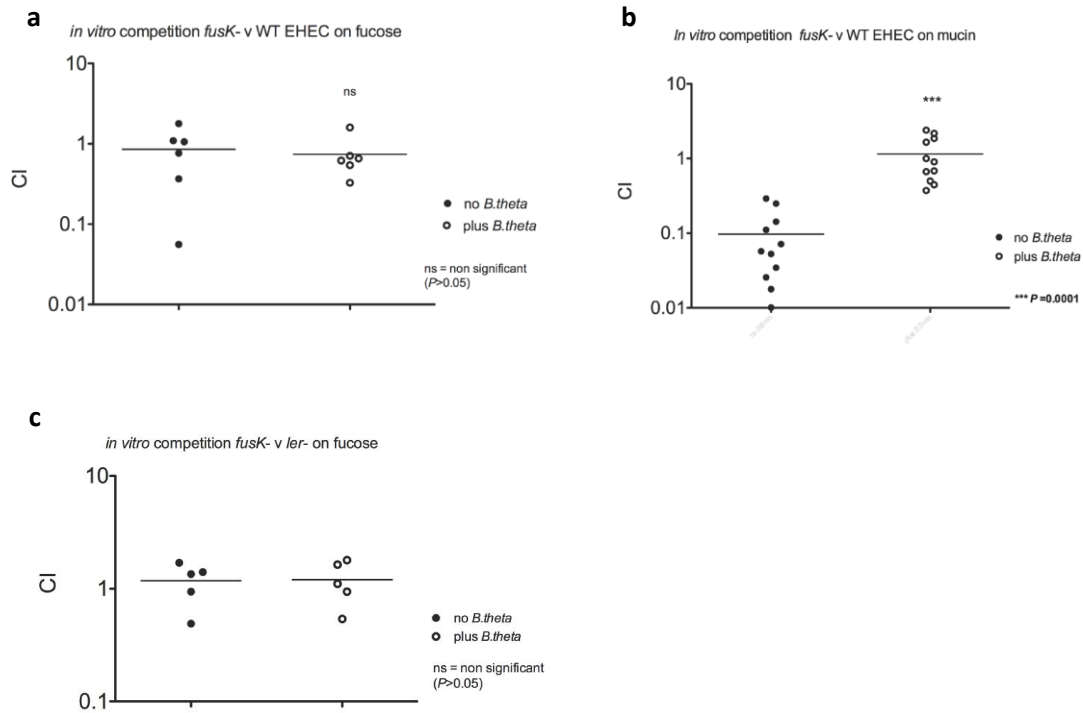
Supplementary Figure 11. EMSA of *LEE1* (*ler*) and *Z0461* regulatory regions with FusR (with acetyl phosphate) showing that FusR does not bind to the *z0461* regulatory region to repress its transcription.



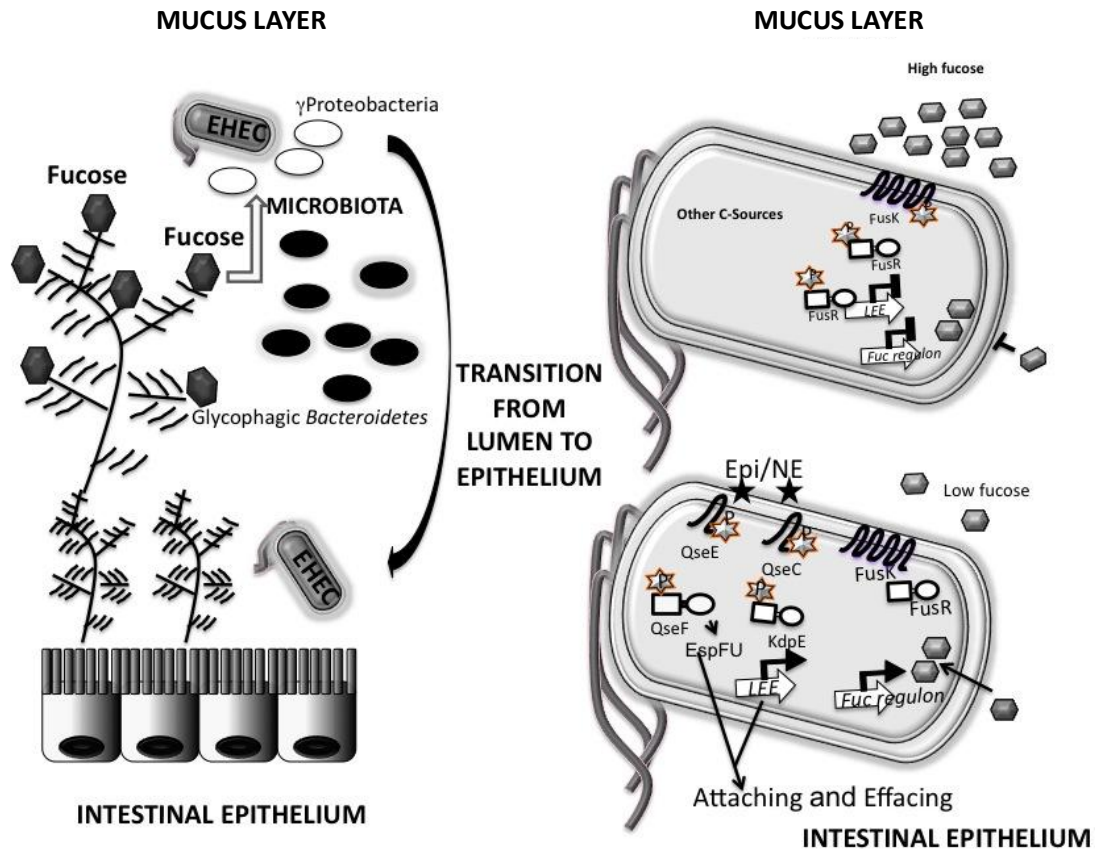
Supplementary Figure 12. Growth of WT and *z0461* mutant in M9 medium with glucose as a sole carbon source. These growth curves reflect 6 independent measurements. ($n=6$ biological samples; significance between generation times calculated through Anova). No significant differences in generation times were observed.



Supplementary Figure 13. EMSA of the *fucPIKUR* promoter with FusR (with acetyl phosphate) showing that FusR does not bind to this promoter region, and that repression of *fuc* expression by FusR is indirect.



Supplementary Figure 14. *In vitro* growth competition assays in the absence or presence of *B. theta*. Competition assays were performed anaerobically at 37°C in DMEM (lacking glucose and pyruvate) with either L-fucose or mucin as sole C-sources in the absence or presence of *B. theta*. Enumeration of EHEC CFUs was performed by plating in McConkey agar in streptomycin (EHEC is streptomycin resistant), enumeration of the *fusK* mutant was performed by also plating in cholamphenicol (*fusK* mutant used in these assays is Cloramphenicol resistant). Competition index (CI) was calculated by dividing the *fusK* CFUs by WT CFUs, or *fusK* CFUs by *ler* CFUs. **a**, Competition between $\Delta fusK$ and WT in DMEM no glucose with fucose. **b**, Competition between $\Delta fusK$ and WT in DMEM no glucose with mucin. **c**, Competition between $\Delta fusK$ and Δler in DMEM no glucose with fucose. ($n=5-11$; three asterisks, $P < 0.0001$; ns, $P > 0.05$, Student's *t*-test).



Supplementary Figure 15. Model of fucose sensing modulating intestinal colonization by EHEC. Fucose available in the mucus layer through *Bacteroidetes* degradation of host glycans inhibits LEE expression through FusKR. In proximity to the epithelial the QseCE adrenergic sensors repress *fusKR* de-repressing LEE expression, and also activate LEE expression through the KdpE RR.

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