Supporting Information

Table S1. Strains and plasmids used in this manuscript; related to Bacterial Strains and Growth Conditions in Methods section

Designation	Strain	Background (resistance)	Purpose	Reference
ZMG01	WT EHEC	86-24 (StrR)	O157:H7 parent strain	Griffin et al., 1988
ZMG02	Δ*argR* EHEC	86-24 (StrR)	*argR* deletion in 86-24	This study
ZMG03	Δ*artP* EHEC	86-24 (StrR)	*artP* deletion in 86-24	This study
ZMG04	Δ*argH* EHEC	86-24 (StrR)	*argH* deletion in 86-24	This study
ZMG05	WT EHEC pACYC184 empty vector	86-24 (StrR CmR TetR)	WT EHEC complementation control	This study
ZMG06	Δ*argR* EHEC pACYC184 empty vector	86-24 (StrR CmR TetR)	Δ*argR* EHEC complementation control	This study
ZMG07	Δ*artP* EHEC pACYC184 empty vector	86-24 (StrR CmR TetR)	Δ*artP* EHEC complementation control	This study
ZMG08	Δ*argR* EHEC pACYC184 *argR*	86-24 (StrR TetR)	Δ*argR* EHEC with *argR* complementation vector	This study
ZMG09	Δ*artP* EHEC pACYC184 *artP*	86-24 (StrR TetR)	Δ*artP* EHEC with *artP* complementation vector	This study
ZMG10	WT *C. rodentium*	DBS770 (CmR)	parent strain	Mallick et al., 2012
ZMG11	Δ*argR C. rodentium*	DBS770 (CmR)	*argR* deletion in DBS770	This study
ZMG12	Δ*artP C. rodentium*	DBS770 (CmR)	*artP* deletion in DBS770	This study
ZMG13	WT *C. rodentium* pACYC184 empty vector	DBS770 (CmR TetR)	WT DBS770 complementation control	This study
ZMG14	Δ*argR C. rodentium* pACYC184 empty vector	DBS770 (CmR TetR)	Δ*argR* DBS770 complementation control	This study

ZMG15	Δ*artP C. rodentium* pACYC184 empty vector	DBS770 (CmR TetR)	Δ*artP* DBS770 complementation control	This study
ZMG16	Δ*argR C. rodentium* pACYC184 *argR*	DBS770 (CmR TetR)	Δ*argR* DBS770 with *argR* complementation vector	This study
ZMG17	Δ*artP C. rodentium* pACYC184 *artP*	DBS770 (CmR TetR)	Δ*artP* DBS770 with *artP* complementation vector	This study
Designation	**Plasmid**	**Background (resistance)**	**Purpose**	**Reference**
pZMG01	pACYC184 empty vector	CmR TetR	Parent complementation vector	NEB, Ipswich, MA
pZMG02	pACYC184 *argR*	TetR	*argR* ORF cloned into CmR cassette of pACYC184	This study
pZMG03	pACYC184 *artP*	TetR	*artP* ORF cloned into CmR cassette of pACYC184	This study
pZMG04	pBAD empty vector	AmR	Parent protein expression vector	NEB, Ipswich, MA
pZMG04	pBAD *argR*	AmR	N-terminally tagged *argR* expression vector	This study

Table S2. Primers used in preparation of this manuscript; related to Bacterial Strains and Growth Conditions in Methods section

Primer	**Sequencing**
ler F	CGAGAGCAGGAAGTTCAAAGTG
ler R	ACACCTTTCGATGAGTTCCG
escC F	CTGAAGACAATGGCAAGTAATGG
escC R	ACTGCATTAAGACGTGGATCAG
escV F	GAGTGCAAAAGGAAAGCCAG
escV R	ATGATACCAGCAATAGCGTCC
tir F	GAGGGAGTCAAATAGCGGTG
tir R	ATCTGAACGAAGGCTGGAAG
eae F	TGGGATGTTCAACGGTAAGTC
eae R	TTTAACCTCAGCCCCATCAC
espA F	AGCTATTTGAGGAACTCGGTG
espA R	CATCTTTTGTGCCGTGGTTG
espB F	GGTCAAGGCTACGGAAAGTG
espB R	TCTTCAGCAAAGTCAGAGGC
Stx2a F	TGTCTGAAACTGCTCCTGTG
Stx2a R	GATATTCTCCCCACTCTGACAC
rpoA RTF	GTGACCCTTGAGCCTTTAGAG
rpoA RTR	ACACCATCAATCTCAACCTCG
infgama F	CAGCAACAGCAAGGCGAAA
infgama R	CTGGACCTGTGGGTTGAC
stx2d F	GTGGATATACGAGGGCTTGATG
stx2d R	CGAACCCGGCCACATATA
slc7a2 F	GTGAAGAGGTTCGGAATCCACA
slc7a2 R	CGTTAAAGCTGCAGAAACCCC
nos2 F	CCTCTTTCAGGTCACTTTGGTAGG
nos2R	TTGGGTCTTGTTCAGCCACGG
Gapdh F	TGTAGACCATGTAGTTGAGGTCA
Gapdh R	AGGTCGGTGTGAACGGATTTG

EHEC-*artP*-LR-F	ATGAGTATTCAATTAAACGGCATTAATTGCTTCTACGGCGCGCATCAGGCG TGTAGGCTGGAGCTGCTTC
EHEC-*artP*-LR-R	TTAGTGAGAGAGATAGTTTTTAAATGCTTCGGTTTGCGGCTCGGTAAAGCC ATATGAATATCCTCCTT
EHEC-*argR*-LR-F	CAATAACTGCCTTAAAAAATTAAAGCTCCTGGTCGAACAGCTCTAAAATCGCTT CATACAGGTCTTTGA
EHEC-*argR*-LR-R	GAGCTAAGGAAGCTAAAATGCGAAGCTCGGCTAAGCAAGAAGAACTAGTTA AAGCATTTAAAGCATT
EHEC-*argH*-LR-F	CAATAACTGCCTTAAAAAATTACCCCAACCGCGCCCTTGCAAAAGCAATCGCC
EHEC-*argH*-LR-R	GAGCTAAGGAAGCTAAAATGGCACTTTGGGGCGGGCGTTTTACCCAGGCAG
Citro-*artP*-LR-F	ATGAGTATTCAATTAAACGGCATTAACTGCTTCTACGGCGCGCATCAGGCGCTGTTCGATA TCACGCTGGATTGCCCGCAGGGCGAAACGCTGGTGTTGCGTGTAGGCTGGAGCTGCTTC
Citro-*artP*-LR-R	TTAGTGAGAGAGATAGTTTTTAAACGCTTCGGTTTGCGGGTTGGCAAAGCAACCTGCATCG CCAAGCTCGACGATATGACCGTTTTCCATATACACGACGCATATGAATATCCTCCTT
Citro-*argR*-LR-F	ATGCGAAGCTCCGCTAAGCAAGAAGAATTAGTAAAGGCGTTCAAAGCGCTCCTTAAAGAA GAAAAATTCAGTTCCCAGGGCGAAATCGTCCTCGCCTTGCCAATAACTGCCTTAAAAAA
Citro-*argR*-LR-R	TTAGAGCTCTTGCTCAAACAGCTCAAGAATGGCTTCGTATAAGTCTT TTACCGTGAAGCCGTTCGCCGGGGTGGTGAAAATGGTGTCGTCGCCA GCAATGGAGCTAAGGAAGCTAAA
EHEC-*artP* F	ATGCCGACATTTATGCTCGCC
EHEC-*artP* R	GCTTCGGTAGCAAAACGAATGGT
EHEC-*argR* F	GACGCAGGCATATTTCTCAATAACG
EHEC-*argR* R	GCGCTAACTGTTTCAGTTGATACT
EHEC-*argH* F	TAACGGTATGCCGATGGGTACG
EHEC-*argH* R	GACTGATAATTGCTCACAGAAACGG
CR-*artP* F	ATGAGTATTCAATTAAACGGCATTAACTGCTTC
CR-*artP* R	TTAGTGAGAGAGATAGTTTTTAAACGCTTCGGT
CR-*argR* F	TTGATAACAATTAATTTACTTTT
CR-*argR* R	ATACAACTACGCTAAATCGCACAAAT
pACYC184 F	TTTTAGCTTCCTTAGCTCCTGAAAATC
pACYC184 R	TTTTTTAAGGCAGTTATTGGTGCCCTTAAACGCCTGG

set1_ler F_-169	GTTGACATTTAATGATAATGTATTTTACACATTAG
set1_ler R_+155	CTCAATTACACTTTGAACTTCCTGCTCTCG
set2_ler F_-372	CCTGTAACTCGAATTAAGTAGAGTATAGTG
set2_ler R_-102	CTATCAAATTAGGACACATCTATTTCATCAAAC
set3_ler F_-575	GGTTACTGTTCAGCTATTTGTCCCTTGTTCC
set3_ler R_-301	GCGTTTCTCTTTATAAGATAGATCTCATTGC
set4_ler F_-493	GTCCCACCTTAGCGCTAAGCACTTTTGAATCAC
set4_ler R_-768	GACGAGTATATATATCTATTAGCTGTTTCCC
set5_ler F_-693	GGAAACTTATTTTGGCCTGTATATAATGTGATG
set5_ler R_-967	GTCCGTTTTACTTGGCATCAGATCATCGCCG
set6_ler F_883	GCATCATATAGTGTCAATAATATACAAG
Set6_ler R_1229	GGCAACTTGAAAAGGGAAAGC
argI F	AACTGCAGCAGGCTGTTGA
argI R	CCTCTTAATGTCCTGCCGAT
Kan F	CCGGAATTGCCAGCTGGGGCG
Kan R	TCTTGTTCAATCATGCGAAACGATCC

Supplementary Figure Legends

Fig. S1. Heat map for non-LEE encoded genes, *in vitro* growth for WT EHEC, select virulence genes for WT and Δ*argR* EHEC in the presence or absence of arginine in high-glucose DMEM. (A) Heat map showing the effect of arginine on non-LEE encoded genes and *recA* from WT EHEC grown in the presence (+arg) or absence (-arg) of 482 μM arginine. (B) *In vitro* growth curve of microaerophilic grown WT EHEC in low-glucose DMEM in the presence (+arg) or absence (-arg) of arginine (482 μM). (C) Western blot analysis of secreted protein EspA from WT, Δ*argR* EHEC. Experiments in C were performed in microaerophilic conditions using high-glucose DMEM with or without arginine, and samples were harvested in late log phase. Error bars represent standard error of mean (SEM). *P< 0.05, **P < 0.01.

Fig. S2. *In vitro* growth for WT, Δ*artP* and Δ*argH* EHEC and respective complement strains. (A) *In vitro* growth curve of microaerophilic grown WT or Δ*artP* or complement EHEC (complemented with *artP* gene on the pACYC184) in low-glucose DMEM with arginine. *In vitro* growth curve of microaerophilic grown WT or Δ*argH* EHEC in low-glucose DMEM with arginine (B) or without arginine (C).

Fig. S3. *In vitro* growth for WT and Δ*argR* EHEC and complement strains. Select virulence genes for WT and Δ*argR* EHEC in the presence or absence of arginine. (A) *In vitro* growth curve of microaerophilic grown or complement EHEC (complemented with *argR* gene on the pACYC184). (B) qRT-PCR analysis to compare the expression of *nleA* and *recA*

genes WT or Δ*argR* in low-glucose DMEM with arginine or without arginine. (C) qRT-PCR analysis to compare the expression of select virulence genes and (D) Western blot analysis of secreted protein EspB from WT, Δ*argR* and cpl EHEC in low-~~glucose~~ DMEM without arginine. (E) Western blot analysis of secreted protein EspB from WT without arginine and Δ*argR* with arginine. Error bars represent standard error of mean (SEM). *P< 0.05, **P < 0.01.

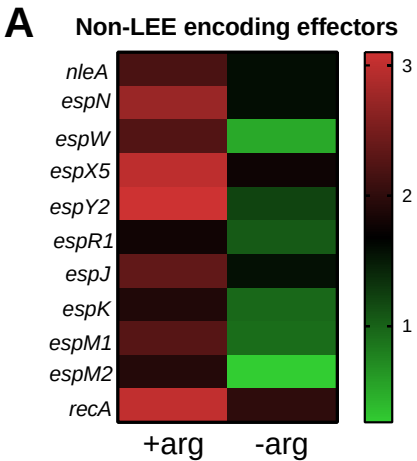
Fig. S4. ArgR is a regulator of LEE. EMSA of His-tagged ArgR and kanamycin probe (negative control), *argI* promoter probe (positive control), and six segments of the EHEC LEE1 regulatory region numbered from the proximal transcriptional start site.

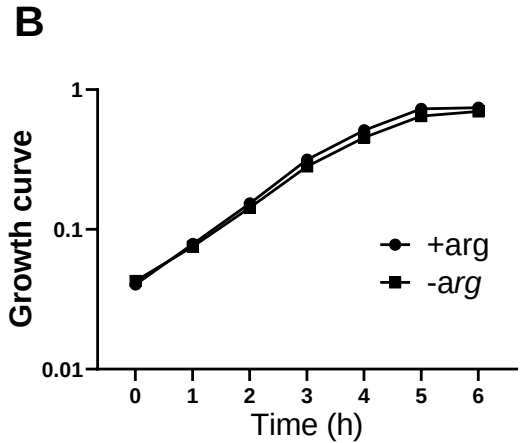
Fig. S5. Arginine induces *Citrobacter rodentium* virulence *in vitro*. (A) qRT-PCR of LEE genes and Shiga toxin 2a, (B) Western blot analysis of secreted proteins EspB and EspA from WT *C. rodentium* grown in the presence (+arg) or absence (-arg) of arginine (482 μM). (C) qRT-PCR analysis to compare the expression of select virulence genes and (D) Western blot analysis of secreted proteins EspB and EspA from WT, Δ*argR* and cpl *C. rodentium* (complemented with *argR* gene on the pACYC184). All experiments were performed in microaerophilic conditions using low-glucose DMEM, and samples were harvested in late log phase. Error bars represent standard error of mean (SEM). *P< 0.05, **P < 0.01, ***P<0.001.

Fig. S6. Δ*ArtP Citrobacter rodentium* strain do not colonize lower intestine of mice. Each symbol indicates the value for an individual mouse 8 days postinfection. (A) Colonization in cecum, colon and stools of mice infected with either WT or Δ *artP C. rodentium*. (B) Expression of *nos2* in colon of mice infected with WT or Δ *artP C. rodentium* or gavaged with PBS. (C)

Survival curve of mice infected with WT or Δ*artP* C. *rodentium.* A total of n=10 mice per group were used for survival curve. (E) Histological score and (F) representation (10X) of colon eight days after WT or *ΔartP C. rodentium* infection. Error bars represent standard error of mean (SEM). *P< 0.05, **P < 0.01, ****P<0.0001.

Fig. S7. The role of dietary arginine in murine *C. rodentium* infection. Each symbol indicates the value for an individual mouse. (A) CFU in stools and (B) arginine levels in colon content of mice in either arginine minus or arginine plus diet infected with WT *C. rodentium* for four days. Error bars represent standard error of mean (SEM). *P< 0.05.


A
Non-LEE encoding effectors
nleA
espN
espW
espX5
espY2
espR1
espJ
espK
espM1
espM2
recA
+arg
-arg
3
2
1


B
Growth curve
1
0.1
0.01
0
1
2
3
4
5
6
Time (h)
+arg
-arg

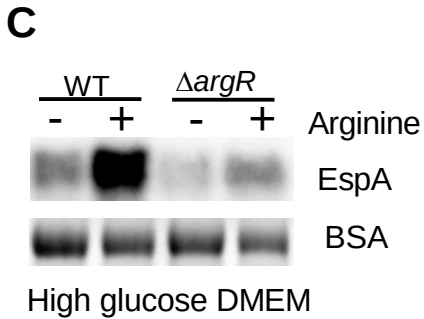

C
WT
ΔargR
-
+
-
+
Arginine
EspA
BSA
High glucose DMEM

Figure S1

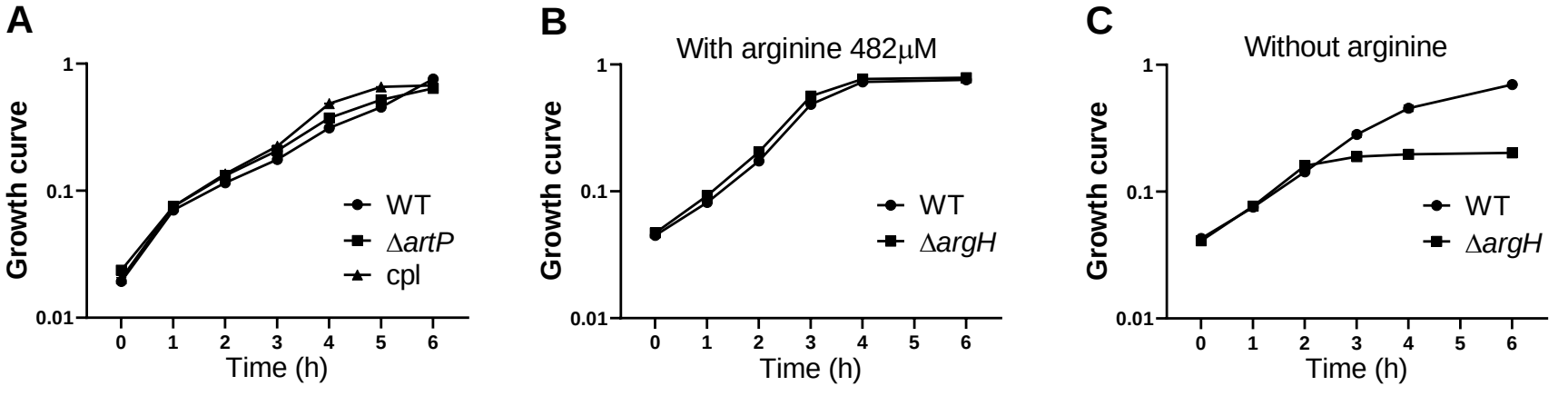

A
Growth curve
1
0.1
0.01
0 1 2 3 4 5 6
Time (h)
WT
ΔartP
cpl
B
With arginine 482μM
Growth curve
1
0.1
0.01
0 1 2 3 4 5 6
Time (h)
WT
ΔargH
C
Without arginine
Growth curve
1
0.1
0.01
0 1 2 3 4 5 6
Time (h)
WT
ΔargH

Figure S2

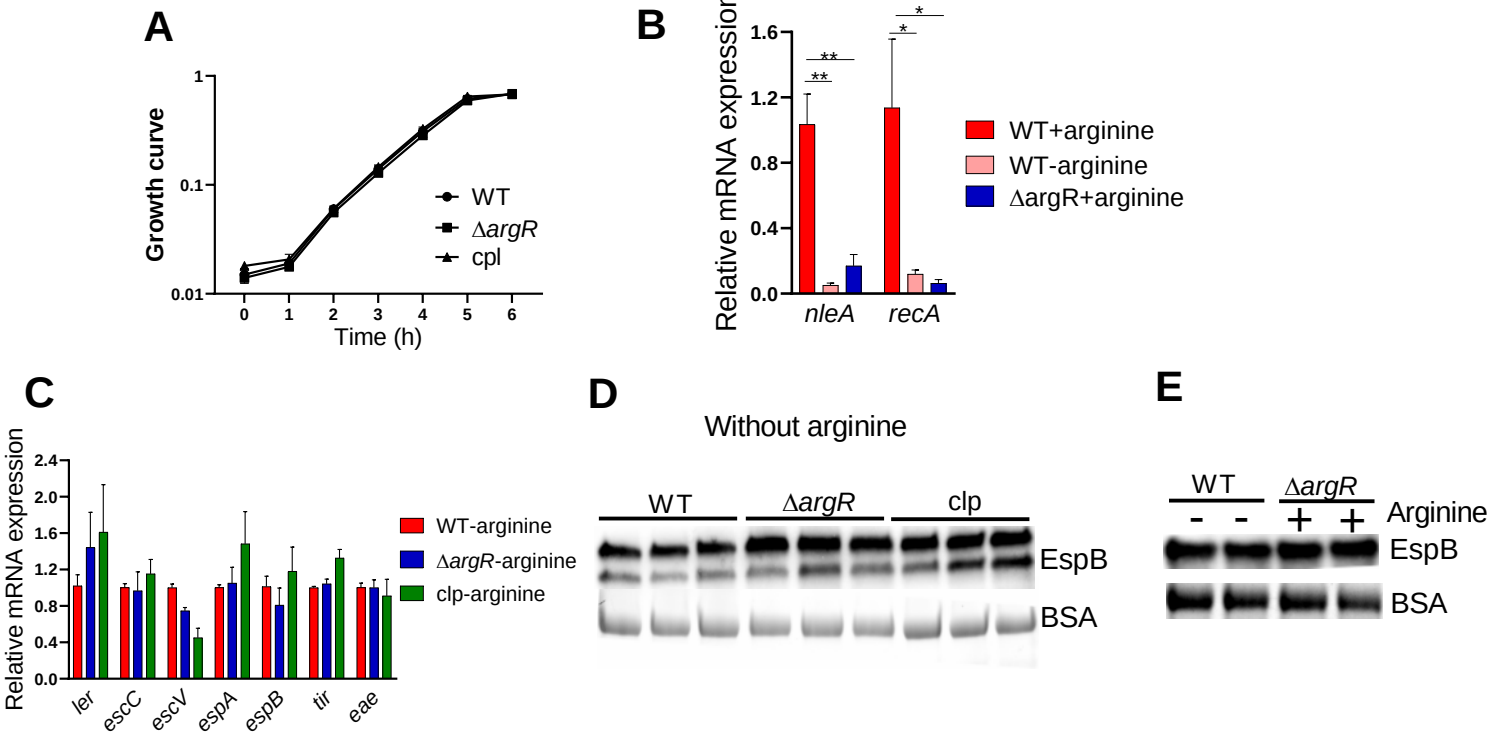

A
Growth curve
1
0.1
0.01
0
1
2
3
4
5
6
Time (h)
WT
ΔargR
cpl
B
Relative mRNA expression
1.6
1.2
0.8
0.4
0.0
**
**
*
*
nleA
recA
WT+arginine
WT-arginine
ΔargR+arginine
C
Relative mRNA expression
2.4
2.0
1.6
1.2
0.8
0.4
0.0
ler
escC
escV
espA
espB
tir
eae
WT-arginine
ΔargR-arginine
clp-arginine
D
Without arginine
WT
ΔargR
clp
EspB
BSA
E
WT
ΔargR
- - + +
Arginine
EspB
BSA

Figure S3

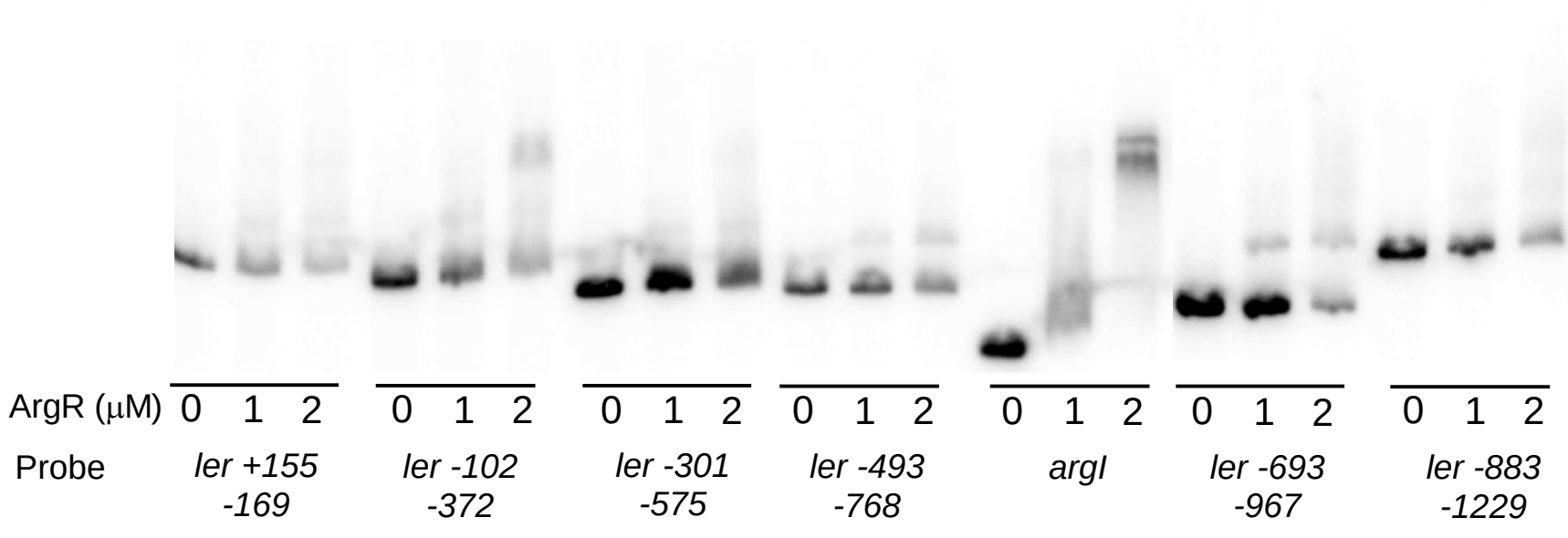

ArgR (μM) 0 1 2 0 1 2 0 1 2 0 1 2 0 1 2 0 1 2 0 1 2
Probe
ler +155 -169
ler -102 -372
ler -301 -575
ler -493 -768
argI
ler -693 -967
ler -883 -1229

Figure S4

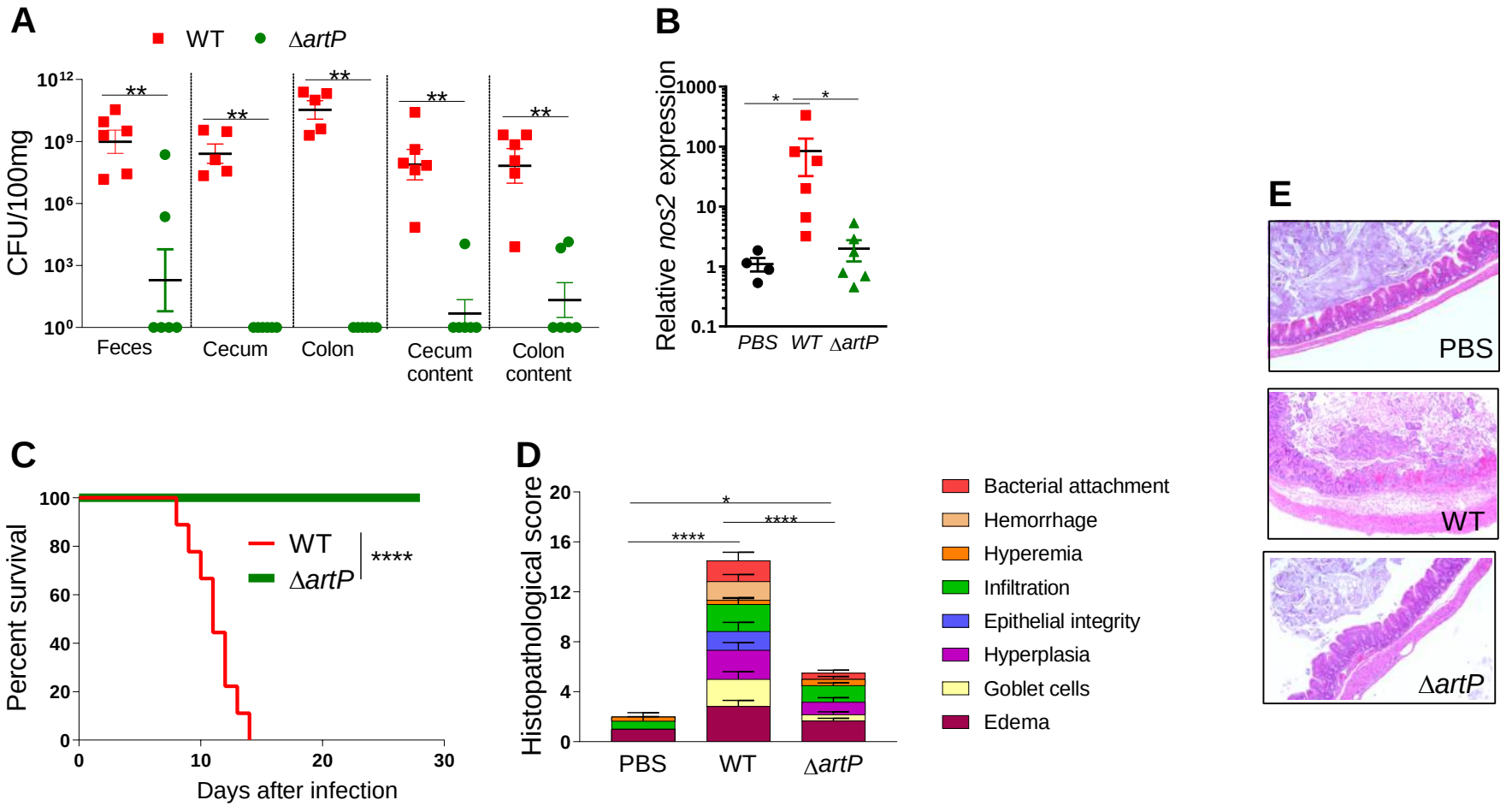

A
WT
ΔartP
CFU/100mg
Feces
Cecum
Colon
Cecum content
Colon content
B
Relative nos2 expression
PBS
WT
ΔartP
C
Percent survival
WT
ΔartP
****
Days after infection
D
Histopathological score
PBS
WT
ΔartP
Bacterial attachment
Hemorrhage
Hyperemia
Infiltration
Epithelial integrity
Hyperplasia
Goblet cells
Edema
E
PBS
WT
ΔartP

Figure S5

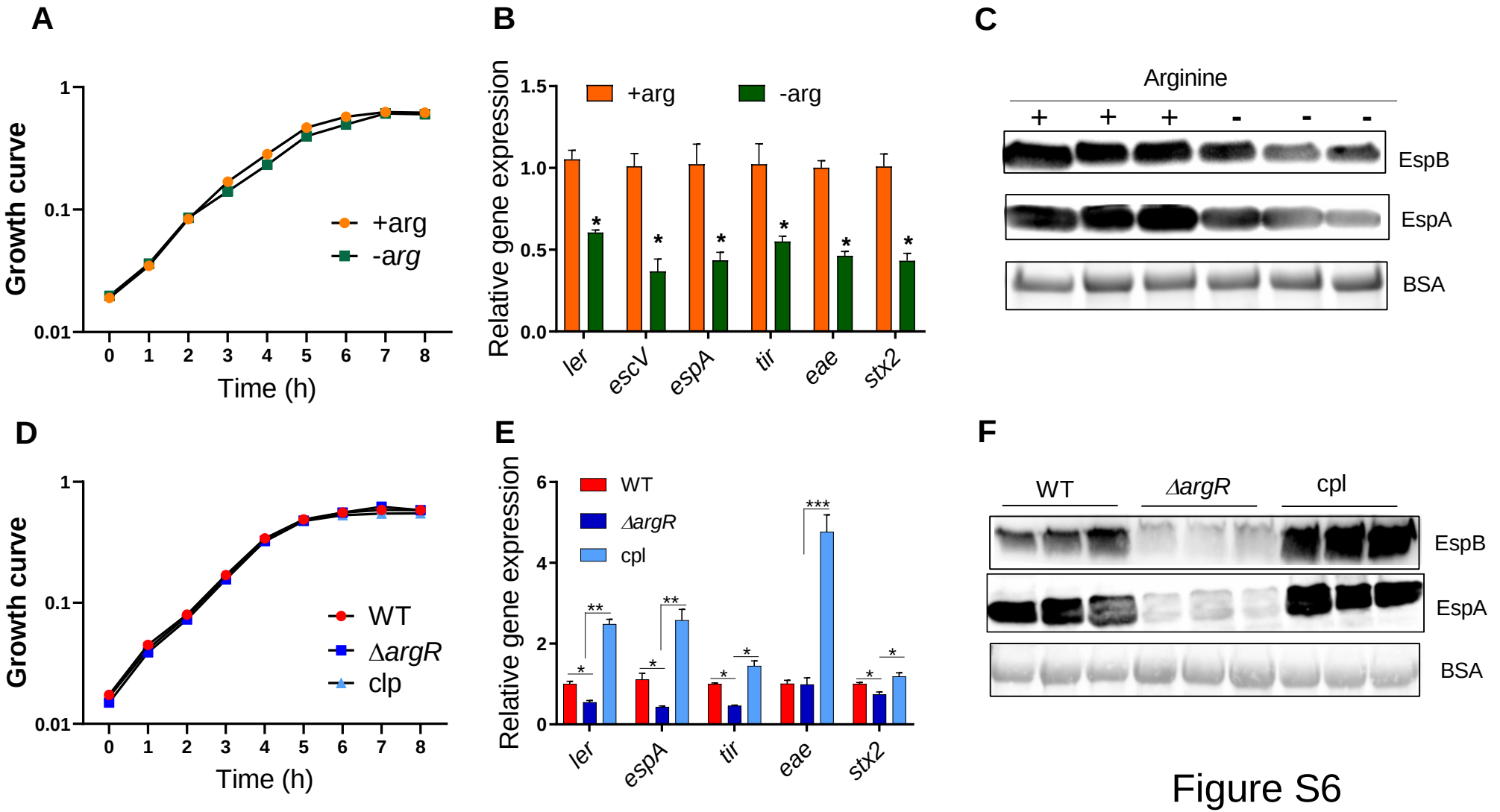

A
Growth curve
1
0.1
0.01
0 1 2 3 4 5 6 7 8
Time (h)
+arg
-arg
B
Relative gene expression
1.5
1.0
0.5
0.0
+arg
-arg
*
ler
escV
espA
tir
eae
stx2
C
Arginine
+ + + - - -
EspB
EspA
BSA
D
Growth curve
1
0.1
0.01
0 1 2 3 4 5 6 7 8
Time (h)
WT
ΔargR
clp
E
Relative gene expression
6
4
2
0
WT
ΔargR
cpl
*
**
***
ler
espA
tir
eae
stx2
F
WT
ΔargR
cpl
EspB
EspA
BSA

Figure S6

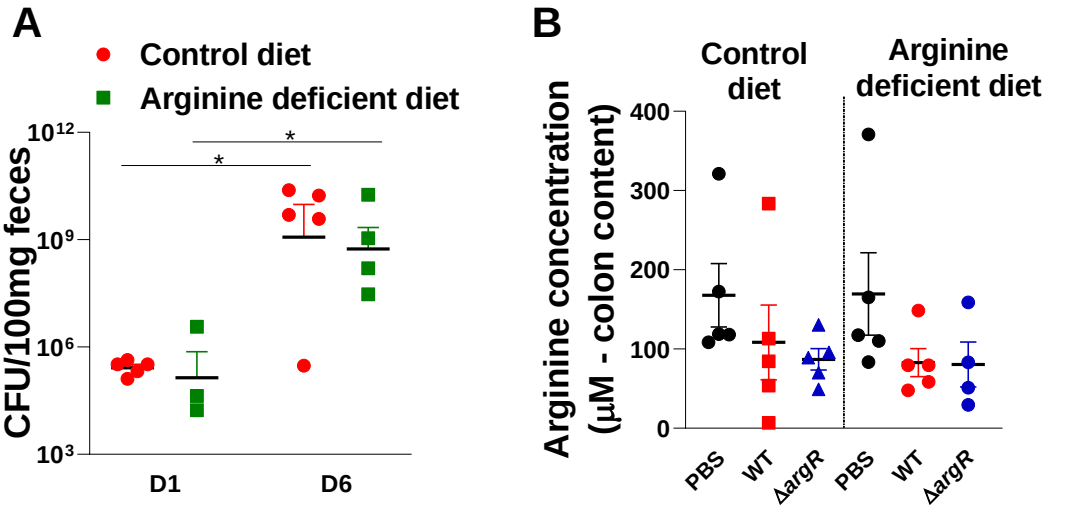

A
Control diet
Arginine deficient diet
CFU/100mg feces
10^{12}
10^{9}
10^{6}
10^{3}
*
*
D1
D6
B
Control diet
Arginine deficient diet
Arginine concentration (μM - colon content)
400
300
200
100
0
PBS
WT
ΔargR
PBS
WT
ΔargR

Figure S7