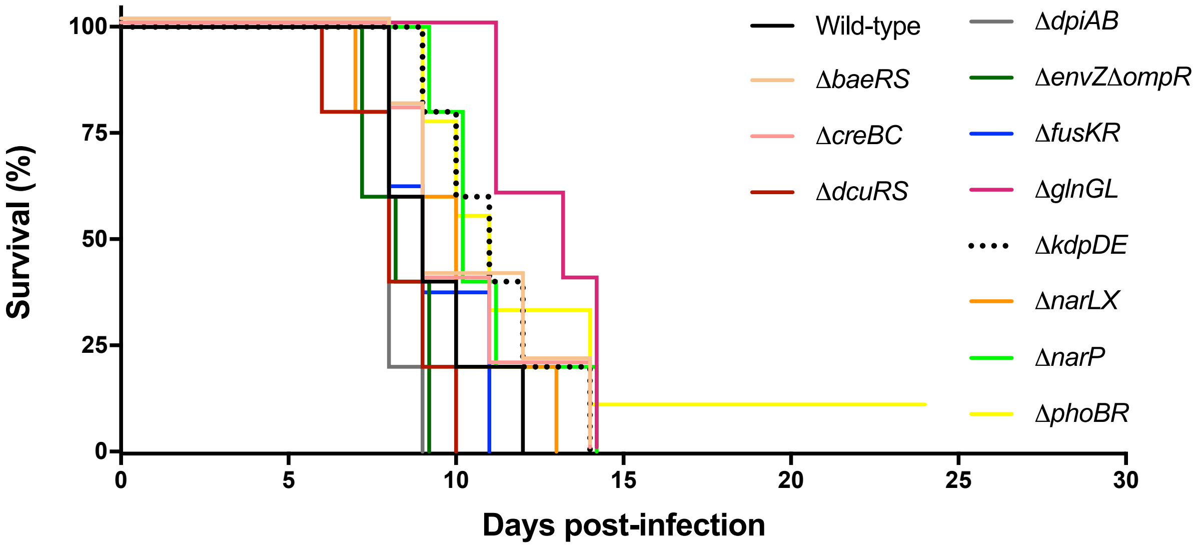

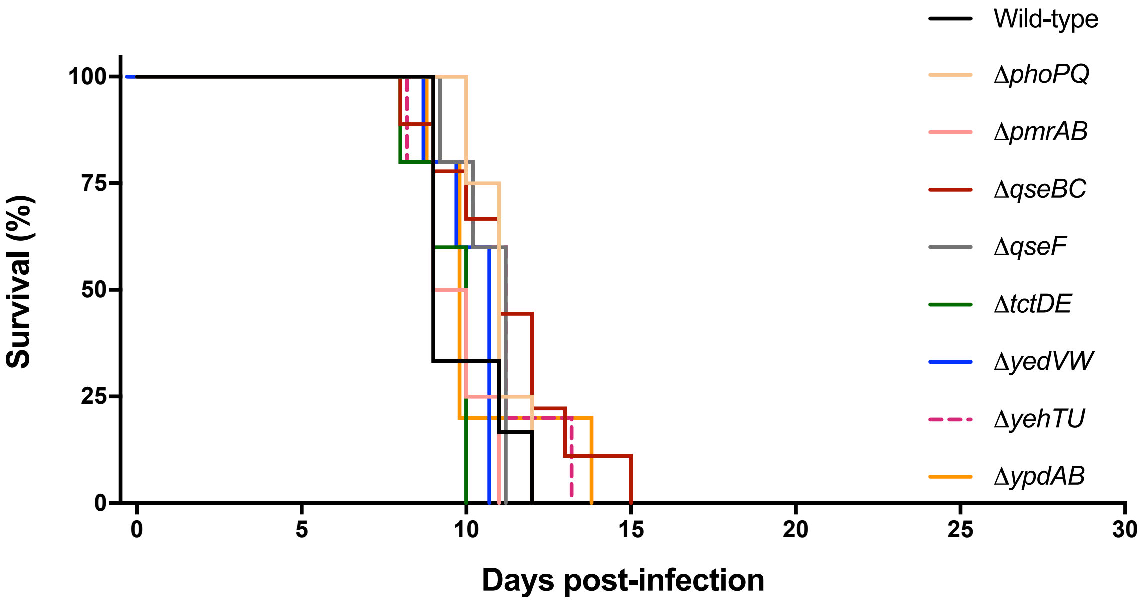

Fig S1. Survival of female C3H/HeJ mice after infection with *C. rodentium*. Animals were orally gavaged 2-3x10^8 CFUs of *C. rodentium* wild-type or a TCS deletion strain. Animals were monitored for 30 days post-infection as described in the materials and methods (n=5-7). The log rank (Mantel-Cox) method was used to test for statistical significance, none were significantly different from wild-type.

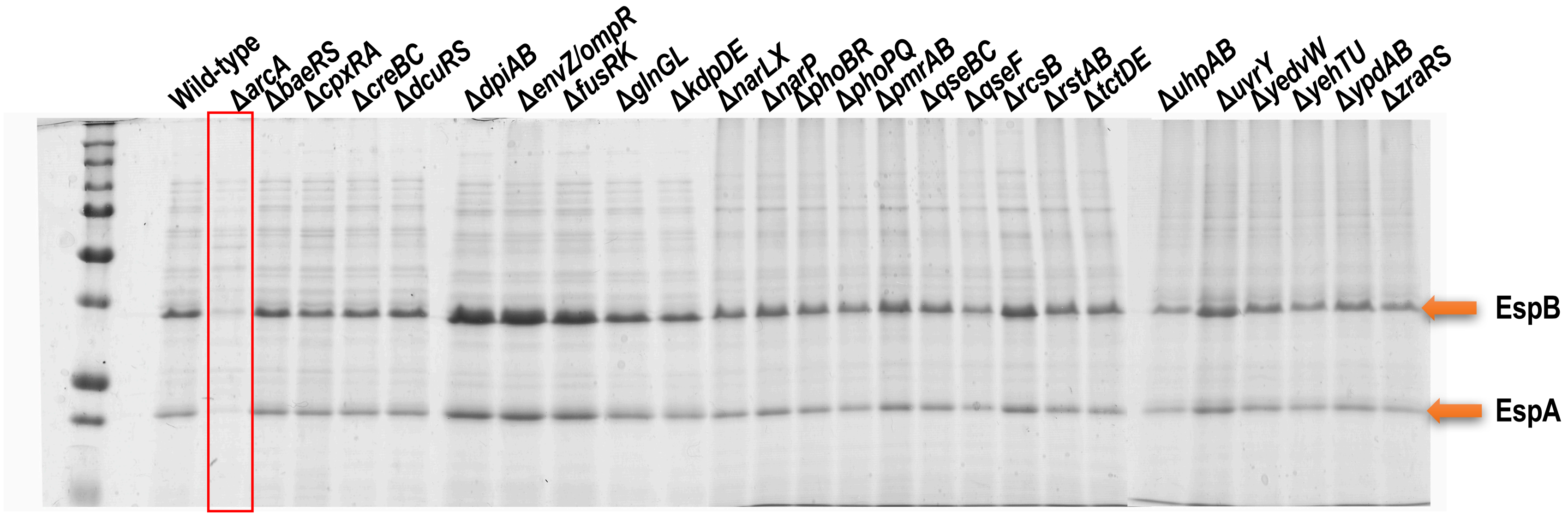

Fig S2. T3S is functional in all TCS deletion strains with the exception of Δ*arcA*. Total secreted proteins of the *C. rodentium* wild-type and TCS deletion strains were prepared, as described in Materials and Methods, and separated on 10% SDS-PAGE gels subsequently stained with Coomassie blue.

TABLE S1 Strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
Citrobacter rodentium		
Wild-type	*C. rodentium* DBS100	(1)
Δ*arcA*	DBS100 Δ*arcA*	This study
Δ*baeRS*	DBS100 Δ*baeRS*	This study
Δ*cpxRA*	DBS100 Δ*cpxRA*	(2)
Δ*creBC*	DBS100 Δ*creBC*	This study
Δ*dcuRS*	DBS100 Δ*dcuRS*	This study
Δ*dpiAB*	DBS100 Δ*dpiAB*	This study
Δ*fusRK*	DBS100 Δ*fusKR*	This study
Δ*glnGL*	DBS100 Δ*glnGL*	This study
Δ*kdpED*	DBS100 Δ*kdpED*	This study
Δ*narLX*	DBS100 Δ*narLX*	This study
Δ*narP*	DBS100 Δ*narP*	This study
Δ*ompR*Δ*envZ*	DBS100 Δ*ompR*Δ*envZ*	This study
Δ*phoBR*	DBS100 Δ*phoBR*	This study
Δ*phoPQ*	DBS100 Δ*phoPQ*	(3)
Δ*pmrAB*	DBS100 Δ*pmrAB*	(4)
Δ*qseBC*	DBS100 Δ*qseBC*	This study
Δ*qseF*	DBS100 Δ*qseF*	This study
Δ*rcsB*	DBS100 Δ*rcsB*	This study
Δ*rstAB*	DBS100 Δ*rstAB*	This study
Δ*tctDE*	DBS100 Δ*tctDE*	This study
Δ*uhpAB*	DBS100 Δ*uhpAB*	This study
Δ*uvrY*	DBS100 Δ*uvrY*	This study
Δ*yedWV*	DBS100 Δ*yedWV*	This study
Δ*yehTU*	DBS100 Δ*yehTU*	This study
Δ*ypdBA*	DBS100 Δ*ypdBA*	This study
Δ*zraRS*	DBS100 Δ*zraRS*	This study
Escherichia coli		

DH5α	HuA2 Δ(*lac*)*U169 phoA glnV44* ϕ80*lacZ*ΔM15 *endA recA hsdR17* (r_M^- m_K^+) *thi-1 gyrA96 relA1*	Invitrogen
DH5αλpir	K-12 F$^-$ ϕ80*lacZ*ΔM15 *endA recA hsdR17* (r_M^- m_K^+) *supE44 thi-1 gyrA96 relA1* Δ(*lacZYA-argF*)*U169* λ*pir*	
χ7213	*thr-1 leuB6 fhuA21 lacY1 glnV44 recA1 asdA4 thi-1* RP4-2-Tc::Mu [-*pir*] Kan^r	(5)
Plasmids		
pRE112	Sucrose-sensitive (*sacB1*) suicide vector; Cm^r	(6)
pΔ*arcA*	Δ*arcA* deletion construct in pRE112	This study
pΔ*baeRS*	Δ*baeRS* deletion construct in pRE112	This study
pΔ*creBC*	Δ*creBC* deletion construct in pRE112	This study
pΔ*dcuRS*	Δ*dcuRS* deletion construct in pRE112	This study
pΔ*dpiAB*	Δ*dpiAB* deletion construct in pRE112	This study
pΔ*fusRK*	Δ*fusRK* deletion construct in pRE112	This study
pΔ*glnGL*	Δ*glnGL* deletion construct in pRE112	This study
pΔ*kdpED*	Δ*kdpED* deletion construct in pRE112	This study
pΔ*narLX*	Δ*narLX* deletion construct in pRE112	This study
pΔ*narP*	Δ*narP* deletion construct in pRE112	This study
pΔ*phoBR*	Δ*phoBR* deletion construct in pRE112	This study
pΔ*qseBC*	Δ*qseBC* deletion construct in pRE112	This study
pΔ*qseF*	Δ*qseF* deletion construct in pRE112	This study
pΔ*rcsB*	Δ*rcsB* deletion construct in pRE112	This study
pΔ*rstAB*	Δ*rstAB* deletion construct in pRE112	This study
pΔ*tctDE*	Δ*tctDE* deletion construct in pRE112	This study
pΔ*uhpAB*	Δ*uhpAB* deletion construct in pRE112	This study
pΔ*uvrY*	Δ*uvrY* deletion construct in pRE112	This study
pΔ*yedWV*	Δ*yedWV* deletion construct in pRE112	This study
pΔ*yehTU*	Δ*yehTU* deletion construct in pRE112	This study
pΔ*ypdBA*	Δ*ypdBA* deletion construct in pRE112	This study
pΔ*zraRS*	Δ*zraRS* deletion construct in pRE112	This study

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TABLE S2 Primers used for qPCR

Gene	Forward primer (5’→3’)	Reverse primer (5’→3’)	Product size (bp)
16S	TGTCTACTTGGAGGTTGTGCCCTT	TGCAGTCTTCCGTGGATGTCAAGA	193
arcA	GCCCGGATGGCGAGCAATAC	CAGCTCGCGACCGGTCATTT	125
arcB	CTATCTGAGCGTGCTGGAGTCAAA	CCAGCTGTTGCAGATGACGAA	119
baeR	CTTGACCTGACTCCCGCCGA	CGATCGGTCACCACCCGGTA	122
barA	CCTGATGGATATTCAGATGCC	CGCCAGATAGTCATTCATACC	163
cpxR	GGAACAGGCGCTGGAGCTTC	ACGGGGGTCTGGTGTGTCTG	120
creB	CGCTGTCGCTTACGCGCTAT	CGGCCCAGACGCTTTCCATC	102
dcuR	TGGCGAATGCGGTGAATATC	ACGTCCCGTTACGCCATAAT	107
dpiA	CAGCGCGAGTCAGCGACAAA	TTCACCGCGTTCAGCGTCAG	108
fusR	GCTTACTCCGCGAGAGCGTG	GGCGCGATACACATGGACCG	112
glnG	TGATTACCTGCCAAAACCGT	TCGGACCGTTGATCTGGATA	113
kdpE	GTCCGGCAGACCGAGATCGA	AGGCCATTCGCCGCTTTCTG	140
narL	GCCAGGCATGAACGGACTGG	CGCCGTCACGACGTCTTCTT	109
narP	CGAACCGTCTCGACCCCGAT	TAATTTGCGCCGTCACGCCA	108
narQ	GTTGCAGATCATCCGTGAAG	CGAATATACACCGTGTGGTTG	111
ompR	GAAACCGTTTAACCCGCGTGAACT	AACTTACCAAAGGCAATCACCGCC	117
phoB	CTCGACATGGGACCGACCGA	TGCACATCGACCGTGCGATC	137
phoP	AGCTGTAACATCAAGGCGTC	GTCGATCAACGATGAGGTGA	111
pmrA	GGATCAGCACCGGCAGGGTA	GCAATGTCTGGAGAGCGGCC	122
qseB	ACTGGTTCACCGACGGTCGT	AATGTCGCGCCCGTCCATTC	104
qseF	GCGACGCAAAAGGGGGTCTT	ACGATAGACTCGCGCCAGCT	128
rcsB	GAAATCGCCAAGAAGCTGAAC	CAGGGTCACAGAAGAGAGATAG	123
rcsC	TGGGGAGAGGCCACGCTATG	TCCGCTGACGACCTGTTGGT	143
rpoD	ATCAAAGCGAAAGGTCGTAGCCAC	CCATCATCACGCGCATACTGTTCA	130
rstA	AGGCGCACCGAACGATTGAC	GCGCGTTTGCGTCTGCATTT	137
tctD	GGAAGATAACCGTGAGCTGG	AACTCTCGCTATGCAACAGG	116
uhpA	CCATCATGCTTTCGGTACAC	CTACAGCGTTTGGAGAGGA	88
uvrY	CGCGAGTTGCAGATTATGCTGAT	TTACACAGGCCATGGCGAATTG	176
yedW	TTTGCCTTACTGCCCGCGAC	TTCTCAACTGCGCGCGTACC	126
yehT	GCCAGGAGCGCAGTAAGCAG	GCGACGTCGTCCATCTGCAA	115
ypdB	ACAGCACAACCGCGTCGATG	GCTTCCACCGCGTGCTCTTT	144
zraR	TTCCCCTGCTGGCTGAGCAT	GGCATTTTCCAGCTCGCGGA	137

wcaA	TTCGGGCGATAAAATCGGTG	GAATTGACCGCGTTATGGGT	151
espA	ATCTTACGGCTGAGTTAAGCG	CGGCTATTATCCACCGTCG	97
espB	TCTCATCTGTCCTGGGGATT	ACTTCAGAGGCGGTATTGAC	124

TABLE S3 Primers used to generate gene deletion strains

Deletion strain	**Primer**	**Sequence (5'→3')**	**Restriction site**
Δ*arcA*	arcA_1	CTAGGAGCTCGACATCCTGAAGCGCATCGA	*SacI*
	arcA_2	CTAGCTCGAGGATAAGAATGTGCGGGGTCT	*XhoI*
	arcA_3	CTAGCTCGAGTAAGCGGTTTACCACCGTCA	*XhoI*
	arcA_4	CTAGTCTAGAGATGACAGGCAGGTACAGCA	*XbaI*
Δ*baeRS*	baeRS_1	CTAGGAGCTCCAGTAAAGGCATGGGCTTGT	*SacI*
	baeRS_2	CTAGGCTAGCAAAATCGCCAGAAACAGCTTG	*NheI*
	baeRS_3	CTAGGCTAGCCGCCTGCAGAATGGCTTAAC	*NheI*
	baeRS_4	CTAGTCTAGACCGGGGTCAGACATAATCAG	*XbaI*
Δ*creBC*	creBC_1	CTAGGAGCTCCTCTGCAGGTGCCACTTGGA	*SacI*
	creBC_2	CTAGGCTAGCCATCTGCTGCATATTTCTTCCC	*NheI*
	creBC_3	CTAGGCTAGCCTTCACCGTCACTTCACATAA	*NheI*
	creBC_4	CTAGTCTAGACAGCAGATACTGCATCGGAT	*XbaI*
Δ*dcuRS*	dcuRS_1	CTAGGAGCTCGTCACTACCTATCTGAGCACC	*SacI*
	dcuRS_2	CTAGGCTAGCCATGGTGGTCCCGGATTGAC	*NheI*
	dcuRS_3	CTAGGCTAGCTGTCAGTAGACGCCAACGGC	*NheI*
	dcuRS_4	CTAGTCTAGAGATCTTTACCGCGGAACCAG	*XbaI*
Δ*dpiAB*	dpiAB_1	CTAGGAGCTCACCATAATGTCGGGAACCGT	*SacI*
	dpiAB_2	CTAGGCTAGCCGGAAATGCTAACTGGCGAAA	*NheI*
	dpiAB_3	CTAGGCTAGCGCATTTATCGCGGGTGATACA	*NheI*
	dpiAB_4	CTAGTCTAGATCCAGACCGCTGCAATACAT	*XbaI*
Δ*fusRK*	fusKR_1	CTAGGAGCTCGGCGGCTGCAGGTTGATAAT	*SacI*
	fusKR_2	CTAGGCTAGCGTCCTCTTCGAGACTGAGTA	*NheI*
	fusKR_3	CTAGGCTAGCCCCACAAATTTGCAACAAACCC	*NheI*
	fusKR_4	CTAGTCTAGAAAGGCCAATCAGCATTTGCG	*XbaI*
Δ*glnGL*	glnGL_1	CTAGGAGCTCCTCCTACAAGCGTCTGGTCC	*SacI*
	glnGL_2	CTAGGCTAGCCATCTGCAGTCTCCTGACAG	*NheI*
	glnGL_3	CTAGGCTAGCGGAATGGAGTAAGCGTTCAC	*NheI*
	glnGL_4	CTAGTCTAGAGAGGTCCAGCGTCTGGTTAA	*XbaI*
Δ*kdpED*	kdpDE_1	CTAGGAGCTCGGCGCCGTTTATCGGGATT	*SacI*

	kdpDE_2	CTAGGCTAGCGTTTCCACCACGCCAACCA	*NheI*
	kdpDE_3	CTAGGCTAGCCCACAGGATGGCGCAATAAT	*NheI*
	kdpDE_4	CTAGTCTAGAGACGATGAAGTACCGTTCAG	*XbaI*
Δ*narLX*	narLX_1	CTAGGAGCTCGAAGCTGATGTTCGCCATAC	*SacI*
	narLX_2	CTAGGCTAGCACCTTCTTCCTTCAGGTTGC	*NheI*
	narLX_3	CTAGGCTAGCTGCATCAGGAACGTATCTTCT	*NheI*
	narLX_4	CTAGTCTAGAAACAGGCGAAACAGTTTGCTT	*XbaI*
Δ*narP*	narP_1	CTAGGAGCTCCAAGTTCCTGTGGGCAACTT	*SacI*
	narP_2	CTAGGCTAGCAGGCATAGTTATCTCCTGAGA	*NheI*
	narP_3	CTAGGCTAGCCAGTGACGAAAACCACGTTG	*NheI*
	narP_4	CTAGTCTAGAGCATCATTCGATATCGCCAC	*XbaI*
Δ*phoBR*	phoBR_1	CTAGGAGCTCAGCGTCCCGATGTAAATGTC	*SacI*
	phoBR_2	CTAGGCTAGCCAGAATACGTCTCGCCATGA	*NheI*
	phoBR_3	CTAGGCTAGCCTGACAGGCGATTGTAATGC	*NheI*
	phoBR_4	CTAGTCTAGATGCACGTAAGCGTGGAGGAT	*XbaI*
Δ*qseBC*	qseBC_1	GCTCTAGACGCGCCGTCTTCTTTGGTCCAGA	*XbaI*
	qseBC_2	GCGATATCGGTGAACCAGTCGACGCAAAAGC	*EcoRV*
	qseBC_3	GCGATATCGGCAATCTGCCGGAGGGAGGATT	*EcoRV*
	qseBC_4	GCGAGCTCCGCGTAGCCGTGGCAGCCTTCTT	*SacI*
Δ*qseF*	qseF_1	CGGGGTACCCTGAGTATCGCCCGGGATTG	*KpnI*
	qseF_2	CTAGGCTAGCGCTTATCATGGCGATACCTC	*NheI*
	qseF_3	CTAGGCTAGCGAGTAGCTCCGCAACATACC	*NheI*
	qseF_4	CTAGTCTAGAGCACAGAGTGAAGGTCTGCG	*XbaI*
Δ*rcsB*	rcsB_1	CTAGGAGCTCGCGAAGTCGACAACCTGCAT	*SacI*
	rcsB_2	CTAGGCTAGCGTTCATGTATAAGGCTACCTTGC	*NheI*
	rcsB_3	CTAGGCTAGCGAGTAATCGCTTTTCGCCGT	*NheI*
	rcsB_4	CTAGTCTAGAGCTGATCAGCATGATGGACG	*XbaI*
Δ*rstAB*	rstAB_1	CTAGGAGCTCTTTACCGATTACGTGGTGGAG	*SacI*
	rstAB_2	CTAGGCTAGCACTTCCGGATCGTCTTCAACA	*NheI*
	rstAB_3	CTAGGCTAGCGATCGGTTTCTCAGGCGATG	*NheI*
	rstAB_4	CTAGTCTAGACTATCTCAGCGAAGAGGAGT	*XbaI*

Δ*tctDE*	tctDE_1	CGGGGTACCGCGGTCTGCTGGAGCTTTT	*KpnI*
	tctDE_2	CTAGGCTAGCGCCAGCTCACGGTTATCTTC	*NheI*
	tctDE_3	CTAGGCTAGCGACGCAGTAACCTTCCTTTTG	*NheI*
	tctDE_4	CTAGTCTAGAGTGATGGCGAGATCAGAATAG	*XbaI*
Δ*uhpAB*	uhpAB_1	CTAGGAGCTCGCCTGATGATGAACATTCAG	*SacI*
	uhpAB_2	CTAGGCTAGCGGTGATCATGATTTTGTCCTGAC	*NheI*
	uhpAB_3	CTAGGCTAGCGCCTAAGGAGCGGCATGTTT	*NheI*
	uhpAB_4	CTAGTCTAGACCTCTTTGTGCGAGCATTCC	*XbaI*
Δ*uvrY*	uvrY_1	GCTCTAGACCGCGGAGTATACCATAAGC	*XbaI*
	uvrY_2	CTCCGCATTCACCAGTTCGTGGTCATCAA	-[a]
	uvrY_3	AACTGGTGAATGCGGAGACGTTAACAAGC	-[a]
	uvrY_4	GCGAGCTCCGTTCGGGAAAGGACCAAAAT	*SacI*
Δ*yedWV*	yedVW_1	CTAGGAGCTCCCACGAACTGGATCGCCAAT	*SacI*
	yedVW_2	CTAGGCTAGCGTTGTCTTCAATCAGTAAAATCTTC	*NheI*
	yedVW_3	CTAGGCTAGCTGAGTGCGGGTTGCGGTCGC	*NheI*
	yedVW_4	CTAGTCTAGACGCTGACCGGATACATCTTATTC	*XbaI*
Δ*yehTU*	yehTU_1	CTAGGAGCTCCGGGTGGTGTCATGGATATT	*SacI*
	yehTU_2	CGGGGTACCGAAACACGCACATTTGCTGAA	*KpnI*
	yehTU_3	CGGGGTACCCGTCGCTATCTGAAAAGTTTG	*KpnI*
	yehTU_4	CTAGTCTAGATGGAGGCTTTCCTCAACGC	*XbaI*
Δ*ypdBA*	ypdAB_1	CTAGGAGCTCGCTTGGGTTAGGCACCAGTA	*SacI*
	ypdAB_2	CTAGGCTAGCCCGCCAGCAGCATAGTAAATA	*NheI*
	ypdAB_3	CTAGGCTAGCGAAGGAGTTCAGGCAGTTAAT	*NheI*
	ypdAB_4	CTAGTCTAGATGATCCCGGACAGCAAGTAT	*XbaI*
Δ*zraRS*	zraRS_1	CTAGGAGCTCGTCAATGGACGTGAGCGTGG	*SacI*
	zraRS_2	CTAGGCTAGCCATACTCTCCTTCGCCTTTC	*NheI*
	zraRS_3	CTAGGCTAGCCGTTAGTTTTGCTCGCGTTC	*NheI*
	zraRS_4	CTAGTCTAGAGGTCGCCGAAAGTCGAAACC	*XbaI*

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[a] This construct was generated by overlap PCR.

TABLE S4 Two-component signaling systems of *C. rodentium* and *E. coli*[a].

Two-component System	Putative Function	*C. rodentium*	*E. coli* K12	EHEC	EPEC
ArcA/ArcB	Anaerobic respiration	+	+	+	+
AtoC/AtoS	Acetoacetate metabolism	-	+	-	+
BaeR/BaeS	Multidrug efflux	+	+	+	+
CpxR/CpxA	Envelope stress response	+	+	+	+
CreB/CreC	Phosphate regulation	+	+	+	+
CusR/CusS	Metal efflux	-	+	+	+
DcuR/DcuS	Response to dicarboxylate	+	+	+	+
DpiA/DpiB	Citrate fermentation	+	+	+	+
EvgA/EvgS		-	+	+	+
FusR/FusK	Fucose sensing	+	-	+	-
GlnG/GlnL	Nitrogen assimilation	+	+	+	+
KdpE/KdpD	Potassium transport	+	+	+	+
NarL/NarX	Nitrogen metabolism	+	+	+	+
NarP/NarQ	Nitrogen metabolism	+	+	+	+
OmpR/EnvZ	Osmotic regulation	+	+	+	+
PhoB/PhoR	Phosphate regulation	+	+	+	+
PhoP/PhoQ	Virulence	+	+	+	+
PmrA/PmrB	LPS modifications	+	+	+	+
QseB/QseC	Quorum sensing	+	+	+	+
QseF/QseE	Amino sugar metabolism	+	+	+	+
RcsB/RcsC/RcsD	Capsule synthesis	+	+	+	+
RstA/RstB	Resistance mechanisms	+	+	+	+
TctD/TctE	Tricarboxylate transport	+	-	-	-
TorR/TorS		-	+	+	+
UhpA/UhpB	Hexose phosphate uptake	+	+	+	+
UvrY/BarA	Carbon metabolism	+	+	+	+
YedW/YedV	Copper homeostasis	+	+	+	+
YehT/YehU	Unknown	+	+	+	+
YpdB/YpdA	Unknown	+	+	+	+
ZraR/ZraS	Response to Zn	+	+	+	+

[a] To identify the TCSs encoded by *C. rodentium*, a BLAST search was performed against the *C. rodentium* genome using the DNA sequence of the *C. rodentium phoP* response regulator. This search identified 26 RR genes and their cognate HK sensors. Orphan RRs (*hnr*) and genes encoding chemotaxis proteins (*cheY* and *cheB*) were omitted. This list is in agreement with databases available online (Prokaryotic 2-Component Systems, P2CS, http://www.p2cs.org and Microbial Signal Transduction database, http://mistdb.com). With few exceptions, the *C. rodentium* genome shares the same TCSs as the prototypical A/E pathogens EHEC EDL933 and EPEC E2248/69.

TABLE S5 Congo red binding to selected *C. rodentium* strains.

	Aerobic		**Anaerobic**	
Strains	**LB**	**YESCA**	**LB**	**YESCA**
Wild-type	+	+	+	+
Δ*arcA*	+	+	+	+
Δ*rcsB*	++	++	++	++
Δ*uvrY*	+	+	+/-	+