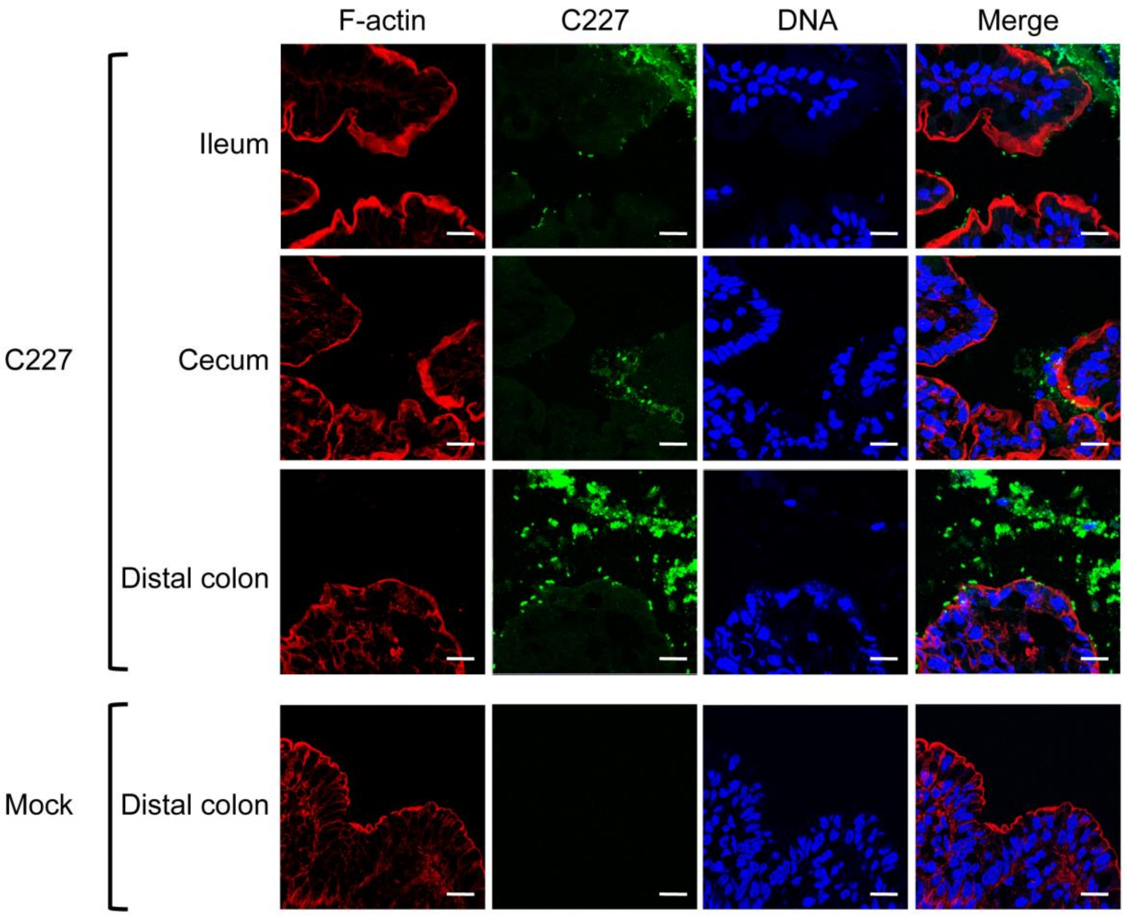
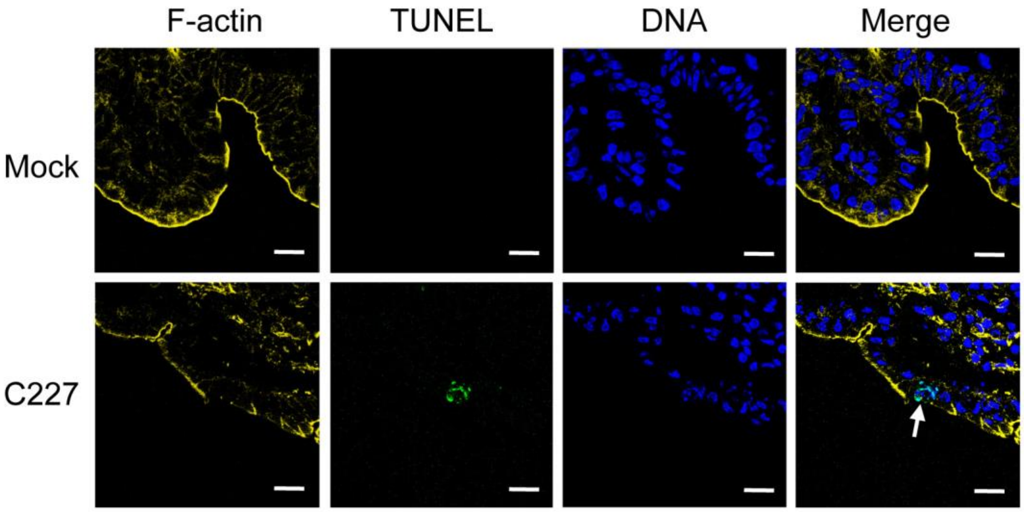
Autotransporters but not pAA are critical for rabbit colonization by Shiga toxin-producing *Escherichia coli* O104:H4

Diana Munera, Jennifer M Ritchie, Stavroula K Hatzios, Rod Bronson, Gang Fang, Eric E Schadt, Brigid M Davis & Matthew K Waldor

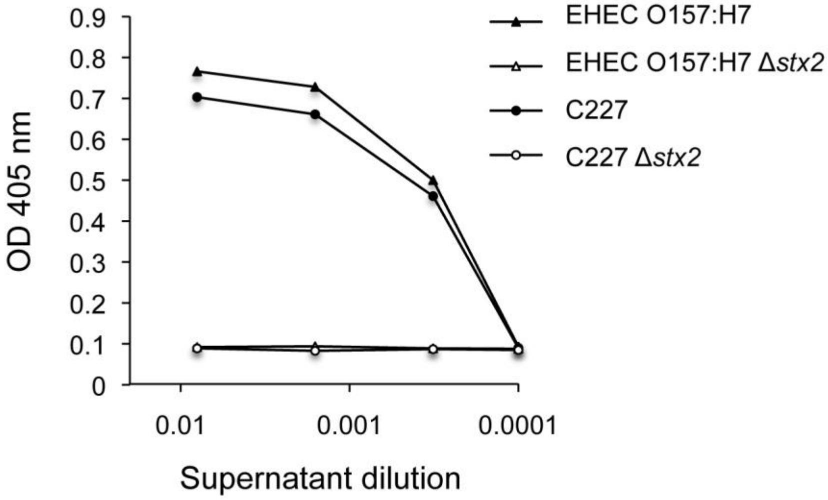
Supplementary Information



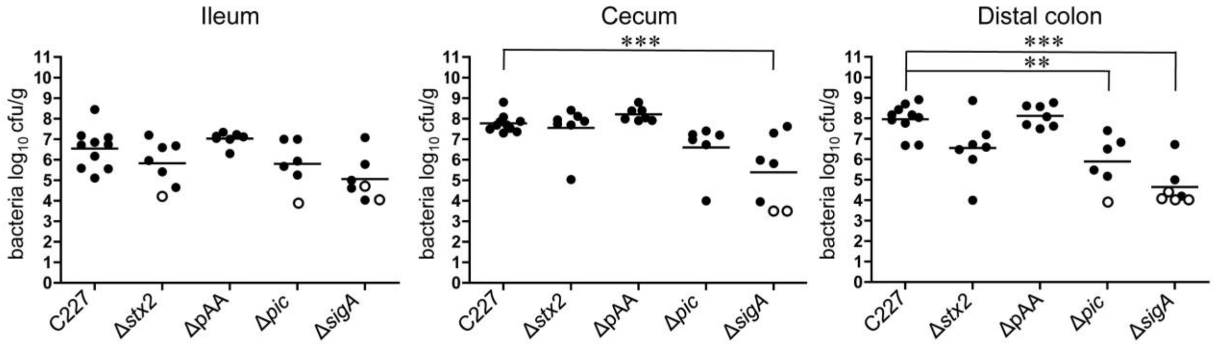
Supplementary Figure 1. Confocal micrographs showing the presence of wild type C227 in different intestinal sections 3 day PI and not in mock infected rabbits. Sections were stained with polyclonal antisera against C227 (green), and counterstained with phalloidin-Alexa 568 (red) and DAPI (blue) to detect F-actin and nuclei, respectively. Scale bar=20 µm.



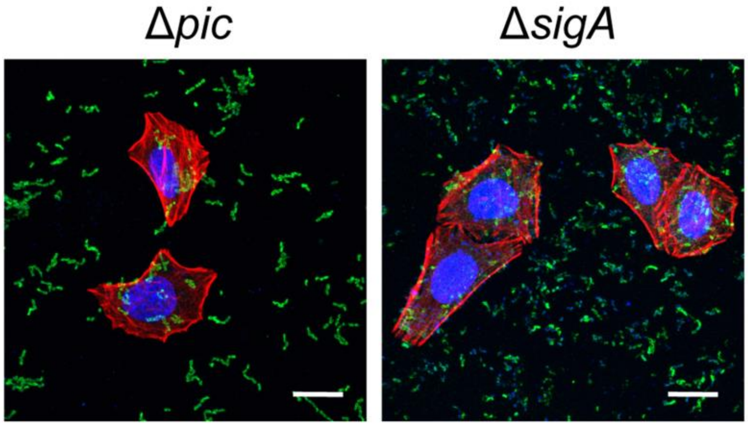
Supplementary Figure 2. Apoptosis in colonic sections from C227-infected rabbits. Sections from distal colon of mock and C227-infected rabbits 3 day PI were stained for apoptotic (TUNEL positive) cells. *In situ* cell death detection kit (Roche) was used according to the manufacturer's instructions, except that the cryopreserved tissue sections were permeabilized for 5 min and the enzyme mixture was applied for 90 min at 37˚C. TUNEL positive cells were detected using a fluorescein-based detection system and apoptotic host cells appear green (arrow). Sections were counterstained with phalloidin-Alexa 633 (yellow) and DAPI (blue) to detect F-actin and nuclei, respectively. Scale bar=20 µm.



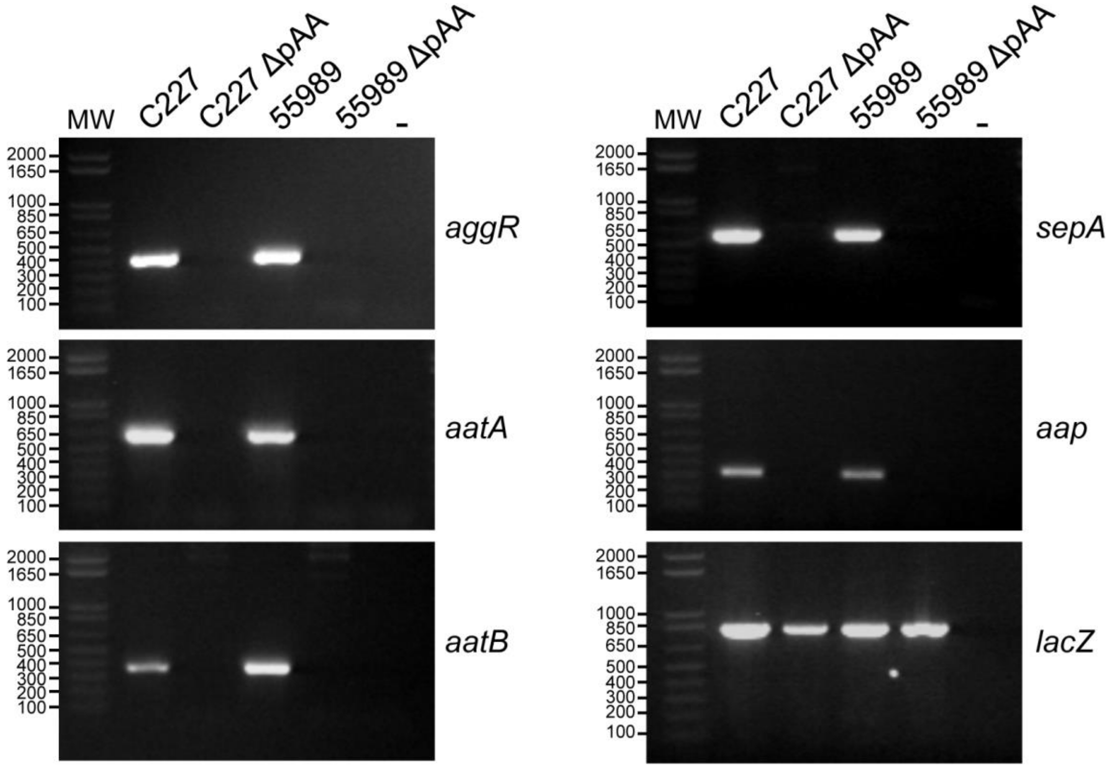
Supplementary Figure 3. ELISA quantification of Stx2 production by C227 and EHEC O157:H7 strains and their isogenic Δ*stx2* derivatives. Overnight cultures of the indicated strain were grown in LB and induced with Mitomycin C (200 ng mL^{-1})[20]. A range of diluted supernatants were added to microtiter plate wells coated with 4D1 antigen[43]. Binding was detected with rabbit polyclonal anti-stx2 antisera (1/5000) followed by anti-rabbit phosphatase conjugated antibody (1/7500). Data represent the average of 2 experiments.



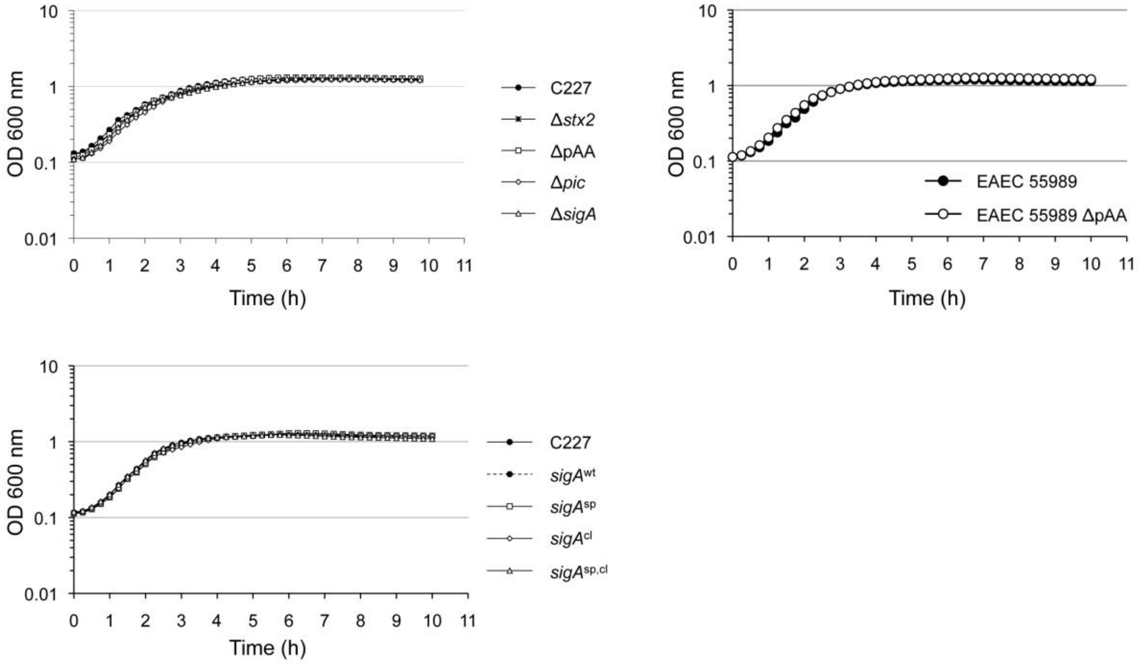
Supplementary Figure 4. Intestinal colonization of infant rabbits by wild type C227 and isogenic mutants 2 day PI. Concentration (cfu g^{-1}) of bacteria recovered from intestinal homogenates of indicated tissues. Data points represent individual rabbits (C227, n=10; Δ*stx2*, n=7; ΔpAA, n=7; Δ*pic*, n=6; Δ*sigA*, n=7). Open symbols represent the limit of detection for samples from which no cfu were isolated. Bars show the geometric mean. Statistical analysis was performed using one way ANOVA and Bonferroni's multiple comparison post-test; ** $P < 0.01$ and *** $P < 0.001$.



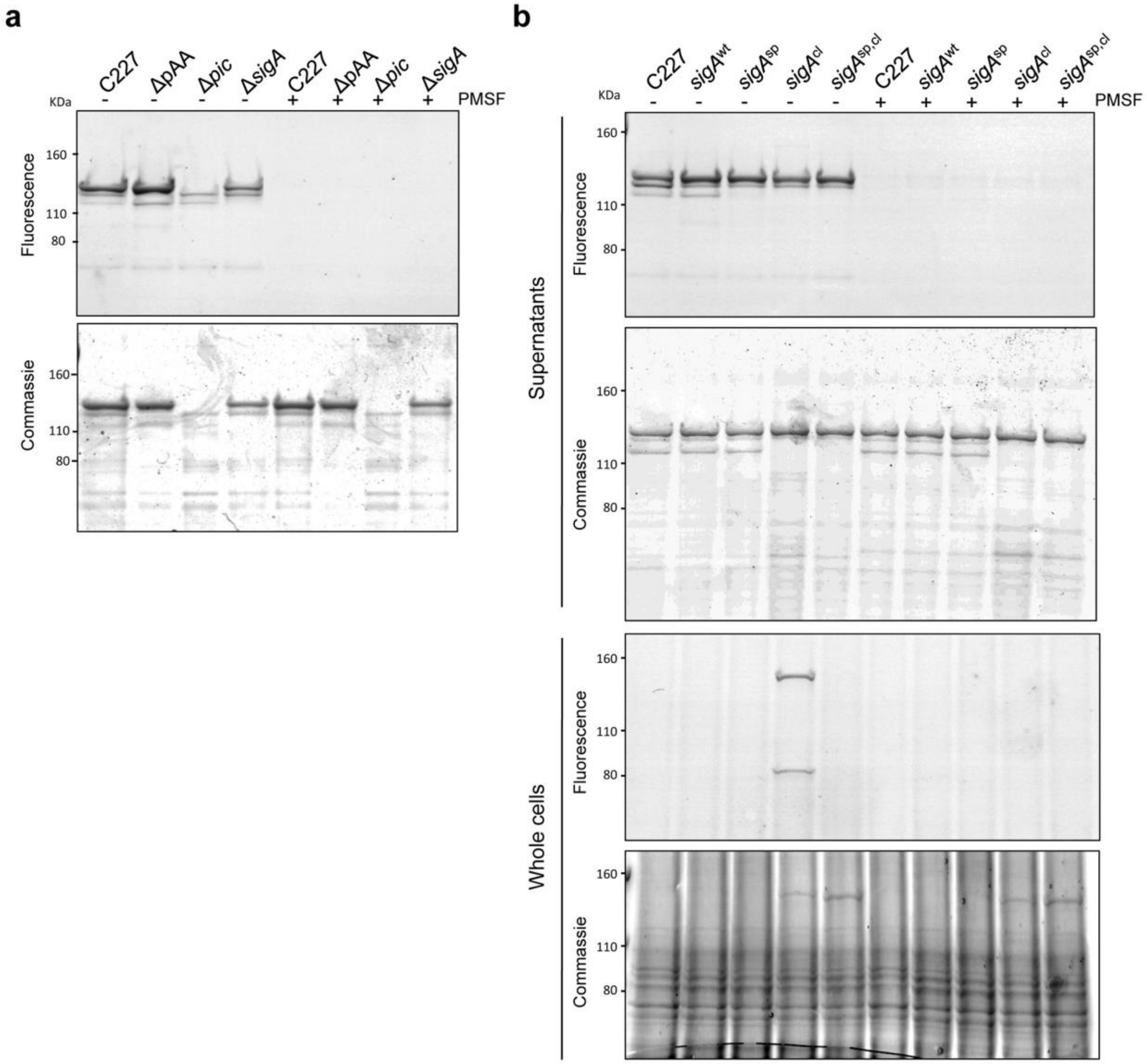
Supplementary Figure 5. C227Δ*pic* and C227Δ*sigA* mutants retain aggregative adherence. HEp-2 cells were incubated with the indicated strain for 3h, then fixed and stained with polyclonal antisera against C227 (green), and counterstained with phalloidin-Alexa 568 (red) and DAPI (blue) to detect F-actin and nuclei, respectively. Scale bar=20 µm.



Supplementary Figure 6. PCR tests of C227 and 55989 strains cured of pAA plasmid. Amplification by PCR of internal fragments of five different genes (*aggR*, *aatA*, *aatB, sepA*, *aap*) encoded in pAA plasmid and chromosomal encoded *lacZ* in O104:H4 C227 and EAEC 55989 strains and their corresponding pAA cured derivatives. Sizes of DNA fragments (bp) in the molecular weight marker lane (MW) are shown on the left.



Supplementary Figure 7. Growth curves of indicated strain in LB media. Each growth curve represents the average of 3 experiments with a standard deviation < 0.02.



Supplementary Figure 8. Activity of SPATEs from C227 and isogenic mutants. (**a**) Activity-based protein profiling of secreted SPATEs from C227 and isogenic mutants. Full fluorescence-scanned blot and coomassie-stained gel corresponding to Fig. 5c. (**b**) Activity-based protein profiling of C227, its **Δ***sigA* derivative complemented with *sigA*-wt, or the indicated *sigA* point mutants. Full fluorescence-scanned blots and coomassie-stained gels from whole cells or supernatants from overnight cultures corresponding to Fig. 5d.

Supplementary Table 1. Differential expression of pAA genes *in vivo* versus *in vitro*

Gene Name	**Product similar to**[a]	**strand**	**start position**	**end position**	***in vitro*** [b] **♯1**	***in vitro*** [b] **♯2**	***in vitro*** [b] **♯3**	***in vivo*** [b] **♯1**	***in vivo*** [b] **♯2**	**log2 fold change**	**log10 p value**	**log10 FDR**	***2 fold change (p value <0.01)**[c]
Q4E6D2	Transposase (IS4 family)	+	2	328	30.7	26.5	36.8	29.8	40.9	0.1	0.1	0.1	
E9Z073	Transposase	+	606	1514	78.3	87.8	89.7	9.0	46.0	-1.7	3.3	2.9	*
B7LWW4	Transposase ORF A, IS1	-	1763	2164	315.2	312.4	286.9	44.8	130.4	-1.9	5.4	4.9	*
B7LWW2	**AggR Transcriptional activator (AAF-III) regulatory protein**	**-**	**2268**	**3062**	**446.7**	**503.1**	**479.9**	**23.6**	**219.8**	**-2.1**	**3.8**	**3.4**	*****
E7JSW2	Integrase core domain protein	-	3233	3600	106.8	147.8	116.6	18.5	98.8	-1.1	1.7	1.5	
B7LFP9	Transposase ORF B, IS629	+	4028	4598	262.6	287.4	312.8	116.7	151.9	-1.1	3.2	2.9	*
Q9S110	Orf57 protein	-	4531	4899	271.5	271.1	317.1	214.1	222.1	-0.4	0.7	0.6	
P71290	Orf58 protein	-	4817	5122	137.9	131.9	159.7	102.7	105.1	-0.5	0.8	0.7	
B7LBU5	Putative reverse transcriptase-like protein	+	5193	5498	73.8	91.5	82.4	71.2	66.5	-0.2	0.3	0.3	
Q9S110	Orf57 protein	-	5574	6008	178.1	164.5	186.7	152.9	109.7	-0.4	0.7	0.6	
P71290	Orf58 protein	-	5926	6231	138.4	140.5	140.5	121.1	68.6	-0.5	1.0	0.8	
B7LB68	Putative reverse transcriptase-like protein	+	6302	7822	535.1	515.7	512.3	433.0	260.7	-0.5	1.3	1.1	
Q327G0	IS629 ORF2	+	7900	8397	76.0	62.8	60.0	47.7	67.7	-0.3	0.2	0.2	
B7LWW0	**Aap Dispersin protein**	**-**	**8595**	**8942**	**6367.9**	**8794.5**	**9074.3**	**76.5**	**276.2**	**-5.6**	**15.8**	**14.6**	*****
P0CE57	Transposase insH for insertion sequence element IS5R	+	9296	10276	122.1	106.8	116.5	76.9	115.3	-0.3	0.3	0.3	
E1PC79	Putative secreted autotransporter toxin sat	+	10498	10737	8.3	6.4	10.4	11.6	14.6	0.5	0.5	0.4	
B7LWV8	Putative uncharacterized protein	+	10854	11069	95.2	139.1	123.1	13.6	14.9	-3.0	11.0	10.1	*
P16943	Insertion element IS630	+	11255	12280	328.4	313.7	298.3	86.7	179.7	-1.3	3.4	3.1	*
B3HJU4	Serine proteAse eata (truncated)	+	12594	14385	981.1	1020.1	1058.0	253.5	444.4	-1.6	4.7	4.2	*
B7LWV1	Putative uncharacterized protein	-	14363	14596	58.6	52.8	51.0	107.1	262.8	1.7	6.1	5.5	*
B7LWV0	Transposase ORF A, IS629	-	14742	14987	73.8	69.1	74.7	189.7	466.8	2.1	9.6	8.8	*
B7LWU9	**AatP permease**	**+**	**15334**	**16494**	**187.3**	**173.2**	**185.3**	**15.7**	**58.4**	**-2.4**	**7.0**	**6.4**	*****
B7LWU8	**AatA outermembrane protein**	**+**	**16494**	**17729**	**314.4**	**321.2**	**287.6**	**20.2**	**67.5**	**-2.9**	**10.8**	**9.9**	*****
B7LWU7	**AatB**	**+**	**17629**	**18447**	**291.2**	**319.8**	**255.9**	**13.8**	**72.3**	**-2.8**	**8.6**	**7.9**	*****
B7LWU6	**AatC ATB binding protein of ABC transporter**	**+**	**18443**	**19069**	**175.0**	**164.4**	**149.3**	**23.7**	**45.7**	**-2.3**	**7.9**	**7.2**	*****
B7LWU5	**AatD**	**+**	**19089**	**20297**	**120.7**	**126.7**	**122.6**	**9.5**	**14.2**	**-3.4**	**12.8**	**11.8**	*****
B7LBV5	Transposase, IS110 family	+	21275	22405	107.0	132.8	104.1	129.6	346.4	1.0	2.5	2.2	*
B7LFP0	Putative uncharacterized protein	-	22521	24086	100.1	91.6	89.0	85.5	110.0	0.0	0.1	0.0	
B7LA97	Putative uncharacterized protein	-	24120	24467	64.0	56.6	60.6	38.2	41.8	-0.6	1.0	0.8	
B7LA98	Putative uncharacterized protein	-	24467	24901	86.3	90.3	75.6	69.7	86.2	-0.1	0.1	0.1	
C6UQD7	Putative uncharacterized protein	+	24997	25194	153.3	143.1	138.7	97.2	156.9	-0.2	0.2	0.2	
B7LBW0	Serine protease pic (ShMu)	-	25495	29586	5061.7	5320.2	5366.3	908.7	1611.7	-2.1	4.4	4.0	*
A7ZH69	Resolvase domain protein	-	29898	30533	72.5	86.5	74.5	17.3	42.0	-1.4	3.0	2.7	*
B7LX00	Putative uncharacterized protein	+	31115	31978	31.3	35.1	35.4	79.0	112.5	1.5	4.8	4.3	*
B7LWZ9	Putative uncharacterized protein	-	32281	32880	20.0	26.2	19.5	29.5	35.5	0.6	0.7	0.6	
D3H5C8	Conjugative transfer protein	+	33211	33591	31.3	27.8	43.9	18.0	64.4	0.1	0.2	0.2	

B7LWZ7	Protein traJ	+	33728	34255	18.3	27.2	11.4	28.4	60.1	1.3	2.0	1.8	
E1U309	Transposase InsAB'	-	34255	34938	91.7	101.5	86.0	105.8	148.6	0.5	0.8	0.7	
D3H556	DNA helicase I (EC 3.6.1.-)	+	34979	39662	207.3	230.8	220.0	259.8	190.7	0.1	0.1	0.0	
B7LWY8	Fertility inhibition protein (Conjugal transfer repressor)	+	40486	41043	217.4	262.2	255.2	62.0	51.9	-2.1	8.7	8.0	*
B7LA98	Putative uncharacterized protein	+	41272	41706	78.3	72.8	73.2	50.6	90.6	-0.2	0.1	0.1	
D3GV17	Transposase	+	41706	41999	41.8	41.4	41.5	11.8	17.1	-1.6	2.7	2.4	*
B7LBX6	Putative uncharacterized protein	+	41950	42261	45.6	49.0	53.1	19.4	42.3	-0.8	0.9	0.8	
D3H551	Transposase	-	42321	43889	813.6	727.8	751.8	75.2	135.4	-2.9	14.2	13.1	*
D3H552	Transposase	-	43912	44256	4.6	3.9	6.5	9.3	11.6	0.9	0.8	0.7	
D3H553	Transposase	-	44259	44933	18.6	17.5	13.8	10.0	19.3	-0.2	0.1	0.1	
Q84A18	Putative uncharacterized protein orf7	+	45002	45250	20.4	9.0	11.2	33.5	94.7	2.0	5.6	5.1	*
P0CE57	Transposase insH for insertion sequence element IS5R	-	45664	46644	121.7	145.4	119.8	89.8	110.2	-0.3	0.6	0.5	
Q79RI5	Negative regulator of repA1 expression (RepA2)	+	46922	47173	150.8	163.1	147.5	147.9	522.2	1.0	2.5	2.2	*
C8URD0	Replication initiation protein RepA1	+	47481	47666	191.6	175.7	154.4	42.2	98.4	-1.4	3.3	3.0	*
C8URD0	Replication initiation protein RepA1	+	47629	48306	86.1	92.9	97.5	96.6	122.1	0.2	0.3	0.3	
B7LWY0	Putative uncharacterized protein	+	49217	49498	234.9	209.6	210.8	56.8	85.3	-1.7	5.6	5.1	*
B7LWX9	Putative uncharacterized protein	+	49501	49773	199.4	182.1	213.7	88.6	70.2	-1.3	4.2	3.8	*
D9Z536	IPF_393	+	49782	50024	230.8	249.7	257.8	96.9	136.6	-1.1	3.1	2.7	*
B1VCJ6	ORF 153 Hypothetical protein	-	49986	50270	257.7	261.0	262.2	113.7	114.2	-1.2	3.8	3.4	*
B7LWX4	Site-specific recombinase	+	50759	51496	60.9	68.9	52.8	25.7	43.2	-0.8	1.4	1.2	
B7LWX3	RepFIB replication protein A	-	51781	52755	1351.7	1401.6	1495.9	170.9	288.6	-2.7	7.7	7.1	*
C8CGL3	Putative uncharacterized protein	-	53068	53553	33.1	33.8	28.5	57.6	116.9	1.4	3.8	3.4	*
D9Z540	VagD	+	54052	54810	413.9	495.6	475.5	52.0	68.0	-2.9	15.8	14.6	*
D8EV54	Putative uncharacterized protein	+	54804	55199	44.5	40.1	30.9	4.4	3.4	-3.3	6.0	5.5	*
B7LX17	Protein ccdA (Protein letA) (Protein H) (LynA) (Modular protein)	+	55269	55544	124.9	124.2	117.3	20.0	18.4	-2.7	10.0	9.2	*
B7LX16	Cytotoxic protein ccdB (Protein letB) (Protein G) (LynB)	+	55522	55851	177.6	155.7	164.1	28.5	22.0	-2.7	11.0	10.1	*
B7LX15	Putative resolvase (Protein D)	+	55855	56658	223.1	164.5	174.0	29.8	40.9	-2.5	9.3	8.5	*
D3H584	Putative uncharacterized protein	+	56807	57481	478.1	450.6	506.4	74.6	109.4	-2.4	11.3	10.4	*
Q9Z4E6	YegA protein	+	57571	57876	243.7	253.5	248.2	61.1	85.7	-1.8	6.5	5.9	*
E9LLV1	Plasmid partitioning and stability protein StbA	+	58239	59270	939.2	1005.1	1038.6	159.6	250.0	-2.3	9.0	8.3	*
Q3ZU22	Plasmid stability protein	+	59266	59679	520.7	535.8	536.4	107.6	131.3	-2.2	10.5	9.7	*
C1J8J5	DNA polymerase V subunit UmuC (UV protection protein)	-	59687	60958	154.2	198.1	169.6	52.5	82.2	-1.4	3.9	3.5	*
A1YLB7	ImpA	-	60961	61398	43.4	37.7	30.9	21.9	53.7	0.0	0.0	0.0	
A1YLB6	Putative resolvase	+	61422	62030	24.7	16.5	20.0	4.6	48.0	0.2	0.3	0.2	
P46004	**AggD Chaperone protein**	**+**	**62882**	**63637**	**1503.3**	**2067.7**	**1861.8**	**29.4**	**105.8**	**-4.8**	**14.8**	**13.6**	*****
B7LX07	**AggC Outer membrane usher protein**	**+**	**63654**	**66179**	**3502.4**	**5614.8**	**5027.0**	**107.6**	**131.3**	**-5.2**	**19.0**	**17.6**	*****
O87745	**AggA protein**	**+**	**66734**	**67234**	**7063.8**	**8167.2**	**8451.0**	**327.7**	**764.0**	**-3.9**	**10.8**	**10.0**	*****
E9XTW5	Integrase core domain-containing protein	+	67374	67691	214.8	228.3	197.0	17.9	61.5	-2.5	8.2	7.5	*

F2W481	ISL3 family transposase	-	67666	67968	36.307	50.004	40.396	9.540	14.171	-1.796	3.368	3.008	*
Q8XC14	Putative IS encoded protein encoded within prophage CP-933O	+	68195	68752	19.309	20.104	21.155	5.260	10.755	-1.396	1.398	1.208	
B7L938	Putative transposase ORF 1, IS66 family	+	68805	70340	148.353	145.584	146.291	57.745	51.645	-1.418	4.243	3.815	*
E3Y4T4	Integrase core domain protein	+	70481	70994	38.846	50.101	47.804	50.160	76.323	0.462	0.673	0.567	
E9Z010	Putative uncharacterized protein	-	70931	71380	55.005	55.238	56.459	64.087	120.254	0.662	1.406	1.214	
E9Z008	Transposase	-	71362	71859	26.829	36.215	28.674	28.897	77.097	0.777	1.127	0.966	
B7LWX0	Putative tail fiber protein	-	71990	72172	4.590	1.381	1.576	17.136	10.363	2.339	3.079	2.742	*
B7LWW9	Putative uncharacterized protein	+	72295	73320	595.797	634.931	677.775	29.652	126.150	-3.156	11.423	10.500	*
B7LWW8	Isopentenyl-diphosphate Delta-isomerase 2 (IPP isomerase 2)	+	73327	73863	418.004	431.719	507.931	22.781	93.542	-3.102	10.789	9.918	*
B1LJZ9	IS5 transposase	+	74681	75328	86.507	60.431	68.255	26.825	79.700	-0.582	0.500	0.416	

[a] putative virulence genes in bold

[b] normalized abundance

[c] we used empirical Bayes estimation and exact tests based on the negative binomial distribution as implemented in the edgeR package[44]

Supplementary Table 2. Bacterial strains used in this study

Strain	Genotype	Source or Reference
C227	Stx2+ EAEC strain serotype O104:H4 C227-11 isolated from German HUS Outbreak	[6]
Δ*stx*2	C227 Δ*stx*$_2$*AB*	This work
ΔpAA	C227 mutant lacking pAA virulence plasmid	This work
Δ*pic*	C227 Δ*pic*; both *pic* loci are deleted	This work
Δ*sigA*	C227 Δ*sigA*	This work
sigA^{wt}	Δ*sigA* mutant complemented with wild type *sigA* in same locus on chromosome	This work
sigA^{sp}	Δ*sigA* derivative complemented with *sigA*-S258A in same locus on chromosome	This work
sigA^{cl}	Δ*sigA* derivative complemented with *sigA*-N1008A, N1009A in same locus on chromosome	This work
sigA^{sp,cl}	Δ*sigA* derivative complemented with *sigA*-S258A, N1008A, N1009A in same locus on chromosome	This work
55989	EAEC 55989 strain serotype O104:H4	[25]
55989 ΔpAA	EAEC 55989 mutant lacking pAA virulence plasmid	This work
EHEC O157:H7	Stx+ EHEC strain serotype O157:H7, EDL933	[45]
EHEC O157:H7 Δ*stx*2	EHEC O157:H7, isolate 905, Δ*stx*$_2$*AB*	[17]
DH5alpha	*supE44* D*(argF-lac)U169 (Δ80dlacΔ (Z)M15) deoR hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Stratagene
MFD*pir*	MG1655 RP4-2-Tc::[ΔMu1::*aac*(3)*IV*-Δ*aphA*-Δ*nic35*-Δ*Mu2::zeo] ΔdapA::(erm-pir)* Δ*recA*	[46]

Supplementary Table 3. Plasmids used in this study

Plasmid	Reference	Primers used for plasmid construction
pDS132	[47]	
pDM4	[48]	
pDS132-flk-stx2	This work	5': Fw-XbaI-Flank-Stx2/ Rv-FlankStx2-BglII and 3': Fw-BglII-FlankStx2/ Rv-FlankStx2-Sac
pDM4-flk-pic	This work	5': Fw-ia-XbaI-Pic1/ Rv-ia-Pic1-BglII and 3': Fw-ia-BglII-Pic2/ Rv-ia-Pic2-SacI
pDM4-flk-sigA	This work	5': Fw-ia-XbaI-SigA1/ Rv-ia-SigA1-BglII and 3': Fw-ia-BglII-SigA2/ Rv-ia-SigA2-Sac2
pDM4-SigA(wt)	This work	Fw-wt-SigA/ Rv-wt-SigA PCR product inserted in pDM4-flk-sigA
pDM4-SigA(S258A)	This work	Fw-wt-SigA/ Rv-S258A-SigA and Fw-S258A-SigA/ Rv-wt-SigA PCR assembled products inserted in pDM4-flk-sigA
pDM4-SigA(N1008A, N1009A)	This work	Fw-wt-SigA/ Rv-N10089A-SigA and Fw-N10089A-SigA/ Rv-wt-SigA PCR assembled products inserted in pDM4-flk-sigA
pDM4-SigA(S258A, N1008A, N1009A)	This work	Fw-wt-SigA/ Rv-S258A-SigA, Fw-S258A-SigA/ Rv-N10089A-SigA and Fw-N10089A-SigA/ Rv-wt-SigA PCR assembled products inserted in pDM4-flk-sigA

Supplementary Table 4. Oligonucleotides used in this study

Primer	Nucleotide sequence (5'- to -3')
5’Fw-XbaI-Flank-Stx2	GATCCTCTAGACAGGTCGCTGGTTCAAATCCAG
5’Rv-FlankStx2-BglII	GGTTATGCCAGATCTATACAGGTGTTCCTTTTGGCTGAAG
3’Fw-BglII-FlankStx2	CACCTGTATAGATCTGGCATAACCTGATTCGTGGTATG
3’Rv-FlankStx2-Sac	CGGGAGAGCTCGAACCCACTCGGGCTTTTTTAC
5’Fw-ia-XbaI-Pic1	AGTACGCGTCACTAGTGGGGCCCTTCTAGACATTGATGTGTTTGCCGGATACA
5’Rv-ia-Pic1-BglII	TCCGGCAGCCCTTTACCCCCACAAGATCTTATGGATTCTCCATGATGTTTAC
3’Fw-ia-BglII-Pic2	GTAAACATCATGGAGAATCCATAAGATCTTGTGGGGGTAAAGGGCTGCCGGA
3’Rv-ia-Pic2-SacI	TAACAATTTGTGGAATTCCCGGGAGAGCTCCCATGGTGGTGAGCGGGGCGCGCT
5’Fw-ia-XbaI-SigA1	AGTACGCGTCACTAGTGGGGCCCTTCTAGAGAATCTGGCCGGTAACGACGGCAG
5’Rv-ia-SigA1-BglII	CGCAAACGCGGCCCGGGCTGTTAAGATCTACGTGAACTCCGATATTTCTGAGT
3’Fw-ia-BglII-SigA2	ACTCAGAAATATCGGAGTTCACGTAGATCTTAACAGCCCGGGCCGCGTTTGCG
3’Rv-ia-SigA2-Sac2	TAACAATTTGTGGAATTCCCGGGAGAGCTCCTGCTGATCCTGCAGGCGCAGTC
5’Fw-wt-SigA	ACTCAGAAATATCGGAGTTCACGTATGAATAAAATTTATTCACTGAAATA
3’Rv-wt-SigA	CGCAAACGCGGCCCGGGCTGTTATCAGAAAGAGTAACGGAAGTTGGC
3’Rv-S258A-SigA	TCATATAGATAAGAACCAGAACCCGCATCGCCAAAAATTGGTGAGT
5’Fw-S258A-SigA	ACTCACCAATTTTTGGCGATGCGGGTTCTGGTTCTTATCTATATGA
3’Rv-N10089A-SigA	GATCCCCCATTCGTTTATTCAGCGCCGCGACTTCTGTCAGGAAGGCT
5’Fw-N10089A-SigA	AGCCTTCCTGACAGAAGTCGCGGCGCTGAATAAACGAATGGGGGATC
5’Fw-aggR	GCAATCAGATTAARCAGCGATACA
3’Rv-aggR	CATTCTTGATTGCATAAGGATCTGG
5’Fw-aatA	CAGACTCTGGCRAAAGACTGTATCAT
3’Rv-aatA	CAGCTAATAATGTATAGAAATCCGCTGT
5’Fw-aatB	ATGGGACTGGACATAGAGTCAAAG
3’Rv-aatB	GATTCGACAATTCATTCCGGTCACC
5’Fw-sepA	AGAAAGGAAATGACCTGAACGCAGG
3’Rv-sepA	TAACCGGTACATTGATGTCACTGG
5’Fw-aap	TTTCTGGCATCTTGGGTATCAGC
3’Rv-aap	TTAACCCATTCGGTTAGAGCAC
5’Fw-lacZ	TTGAACTGCACACCGCCGAC
3’Rv-lacZ	AAATCACCGCCGTAAGCCAACCAC

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