Supplementary information

Figure S1. Growth of *E. coli* EC93 strains in the presence of proteinase K. *E. coli* EC93 strains were cultured in TB medium at 37 °C with shaking and growth monitored by measuring the optical density at 600 nm (OD). Where indicated, the culture medium was supplemented with 20 μg mL^{-1} of freshly prepared proteinase K.

Figure S2. *E. coli* EC93 Δ*cdiA* mutants do not have growth or motility defects. A) *E. coli* EC93 *cdiA*$^+$ and Δ*cdiA* mutants were cultured in tryptone broth supplemented with 0.5% glycerol at 37 °C with shaking and growth monitored by measuring the optical density at 600 nm. **B**) *E. coli* EC93 *cdiA*$^+$ and Δ*cdiA* mutants were seeded at the center of 0.4% agar swim plates and incubated at 37 °C for 12 h. **C**) Cell mobility was measured as the radius of migration in cm and the average ± SEM presented. **D**) *E. coli* EC93 *cdiA*$^+$ and Δ*cdiA* mutants were harvested from biofilms after 2 h of incubation on polystyrene plates and total RNA isolated for real time-quantitative PCR analysis of *fliC*, *pgaA, bcsA* and *wcaA* transcript levels. The average ± SEM is presented for three independent replicates.

Figure S3. Forward scatter analysis of strains expressing CdiA^{EC93} truncation and deletion constructs. Strains CH9604 (*bamA*^{Eco}) and CH9591 (*bamA*^{ECL}) that carry pTNC-WEB (CDI$^-$) or the indicated CDI expression constructs were grown in LB medium and analyzed by flow cytometry for forward scatter.

Figure. S4. Alignment of CdiA^{EC93} and CdiA^{E264} proteins. CdiA^{EC93} (Uniprot: Q3YL96) and CdiA^{E264} (Q2SV12) were aligned using Clustal Omega on the Uniprot web server (http://www.uniprot.org/align/) and the alignment rendered with Jalview. The TPS transport domains are indicated for each protein. The VENN and ELYN peptide motifs that demarcate the toxin CdiA-CT regions are depicted in red font.

Figure S1

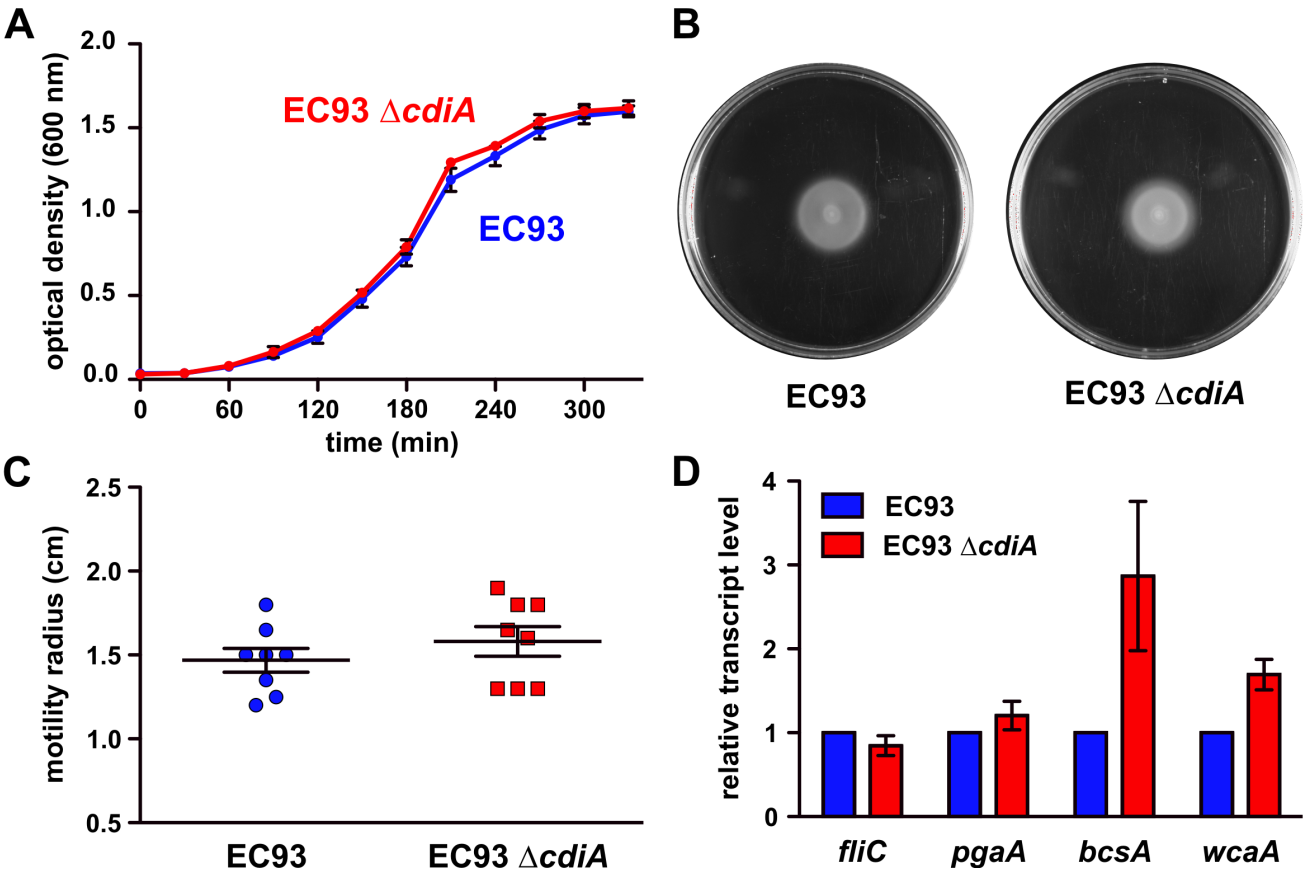
A
optical density (600 nm)
2.0
1.5
1.0
0.5
0.0
0
60
120
180
240
300
time (min)
EC93 ΔcdiA
EC93
B
EC93
EC93 ΔcdiA
C
motility radius (cm)
2.5
2.0
1.5
1.0
0.5
EC93
EC93 ΔcdiA
D
relative transcript level
4
3
2
1
0
EC93
EC93 ΔcdiA
fliC
pgaA
bcsA
wcaA

Figure S2

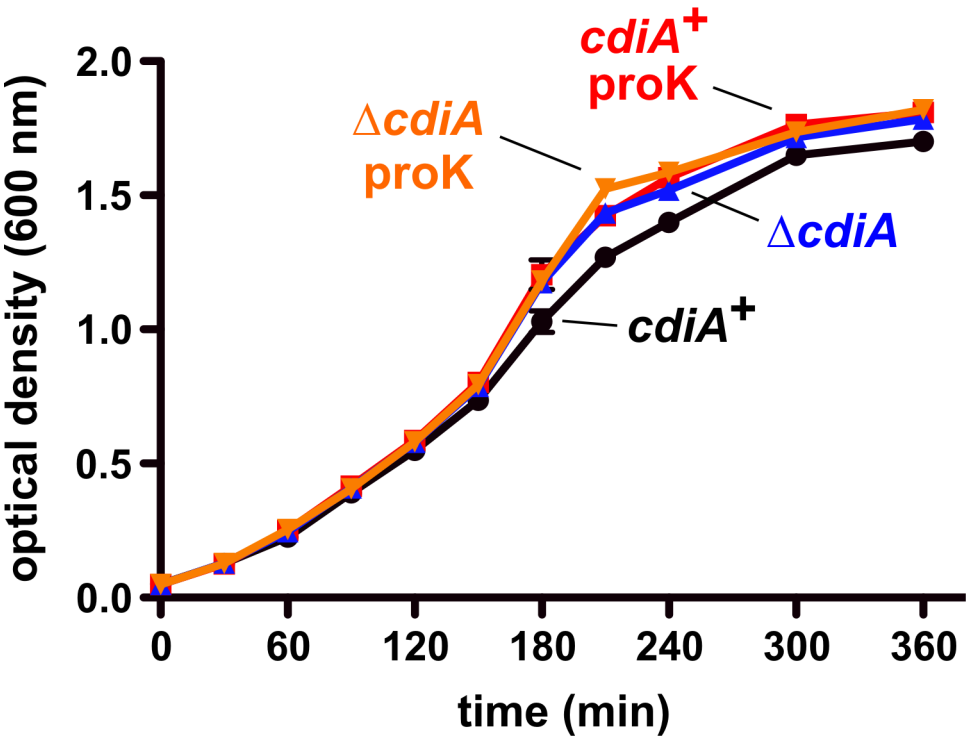
cdiA+
proK
ΔcdiA
proK
ΔcdiA
cdiA+
optical density (600 nm)
2.0
1.5
1.0
0.5
0.0
0
60
120
180
240
300
360
time (min)

Figure S3

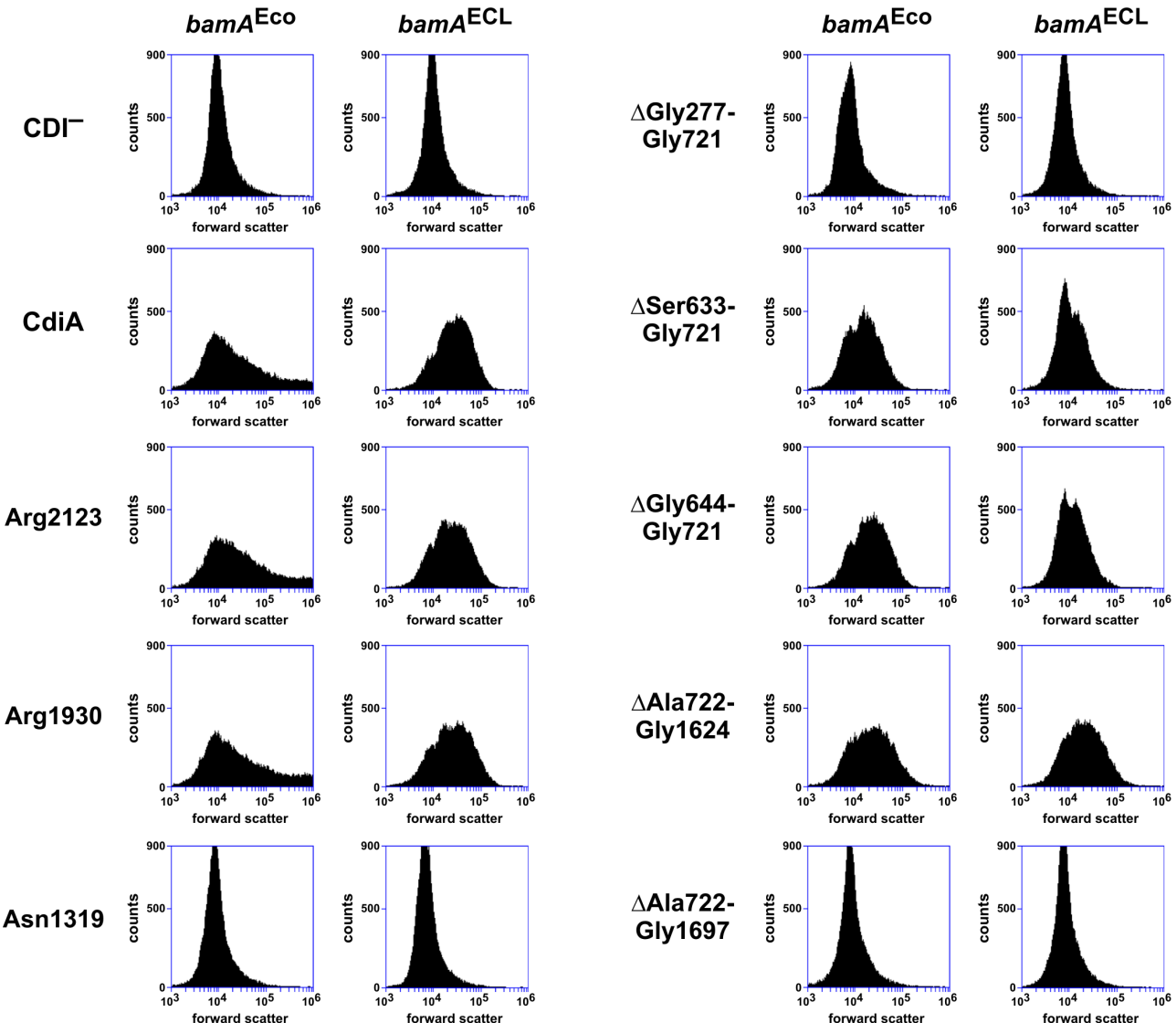
bamA^Eco
bamA^ECL
CDI⁻
CdiA
Arg2123
Arg1930
Asn1319
ΔGly277-Gly721
ΔSer633-Gly721
ΔGly644-Gly721
ΔAla722-Gly1624
ΔAla722-Gly1697
counts
forward scatter

Figure S4

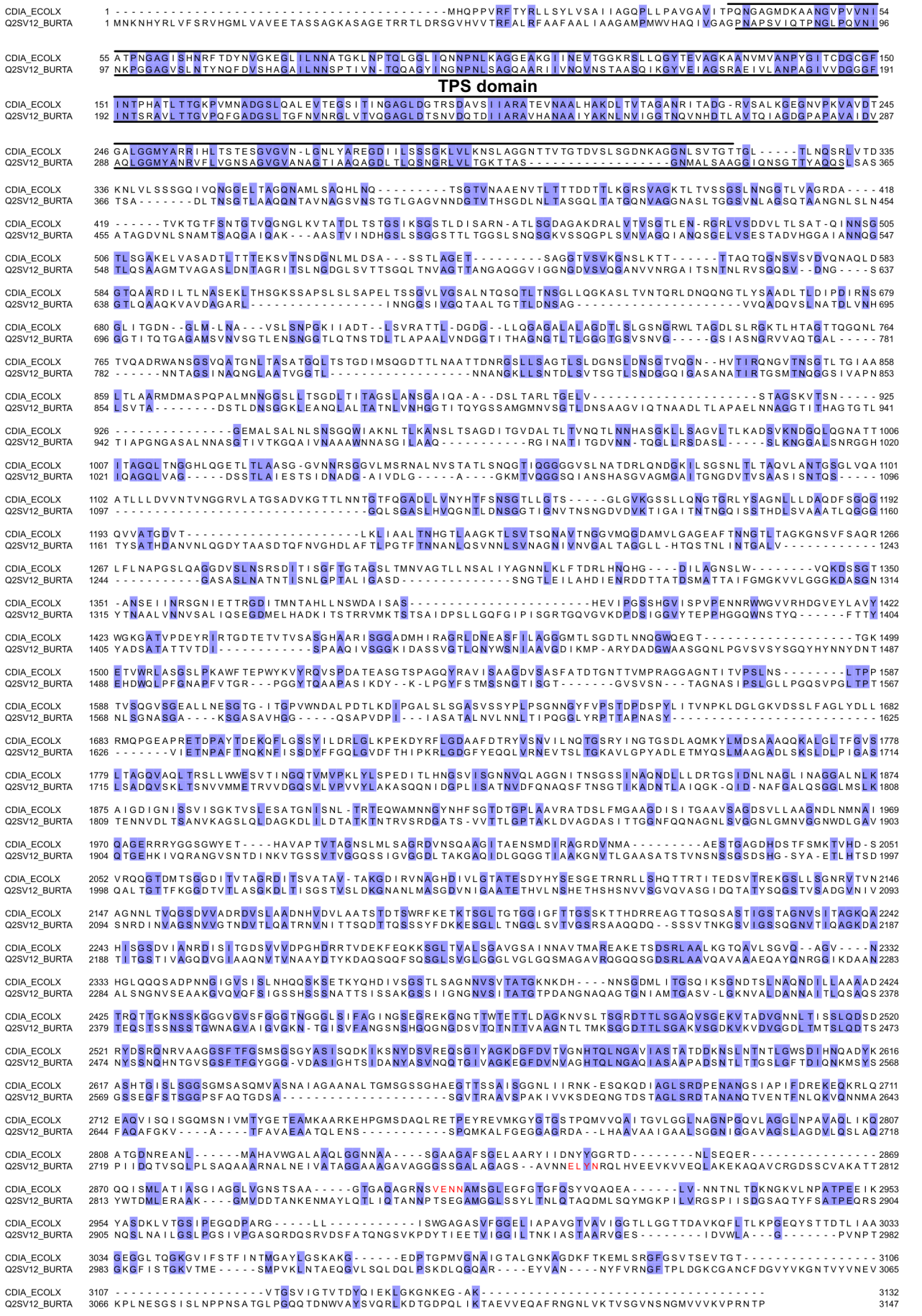

Table S1. Oligonucleotides

Oligonucleotide	***Description***[a]	***Reference***
ΔcdiA-dn-Eco	5′ - TTT GAA TTC TAA TAA GGA AGG GGC AAA ATG AAG	(Ruhe *et al.*, 2013)
ΔcdiA-dn-Xho	5′ - TTT CTC GAG CGT CAG ATC TTT ACC GAT ATT CG	(Ruhe et al., 2013)
ΔcdiA(CT)-up-Sac	5′ - TTT GAG CTC GAT GAA AGC AGC CAG GAA AG	This study
ΔcdiA(CT)-up-Bam	5′ - TTT GGA TCC TAT GCA TTA TTC TCA ACC GAG TTC	This study
EC93-cdiI(KO)-Sac	5′ - TTT GAG CTC AGG AAA TTA AGT ACG CCT CTG ATA AGC	This study
EC93-cdiI(KO)-Bam	5′ - TTT GGA TCC TAT TTT GCC CCT TCC TTA TTA CCT TTT C	This study
EC93-cdiI(KO)-Eco	5′ - TTT GAA TTC GTT GTT GTT GAG AAT AAC TCG CTG AG	This study
EC93-cdiI(KO)-Xho	5′ - TTT CTC GAG TCC AGT ATT TTC CAC TAC CAT CTG G	This study
gfp(mut3)-Bam-for	5′ - TTT GGA TCC AAG GAG ATA TAC ATA TGA GTA AAG GAG AAG AAC TTT TCA CTG G	This study
sfGFP-rev-Bam	5′ - TTT CTG CAG GAT CCT TAT TTG TAG AGC TCA TCC ATG CC	This study
dsRed-Eco-for	5′ - TAA GAA TTC CAC ACA GGA AAC AGC	This study
dsRed-Xho-rev	5′ - TCG CTC GAG CTA CTG GAA CAG GTG G	This study
EC93cdiA-V33-Spe-for	5′ - TTT ACT AGT GTC ATC ACC CCA CAA AAC G	This study
EC93cdiA-G285-Xho-rev	5′ - CAG CTC GAG TTA TCC GGA ACT GCT CAG	This study
pgaA-for	5′ - CAG AAA AAG GTG CCC GAA AAC C	This study
pgaA-rev	5′ - GCA ATC TGT AAC CAG TCA GCA ATT TGG	This study
wcaA-for	5′ - CTG GAA CCG CCA ACA ACT GG	This study
wcaA-rev	5′ - AGC ATA ATC GCC TGG TTA CGT ACC	This study
bcsA-for	5′ - TGA CCC GGT GGT TGC TTA TCC	This study

bcsA-rev	5′ - GCG AGG CGT TGA TAT GCG G	This study
fliC-for	5′ - GCA CAA GTC ATT AAT ACC AAC AGC CTC	This study
fliC-rev	5′ - GCG CTG TTA ATA CGC AAG CCA G	This study
lac-Sac-for	5′ - TTT GAG CTC GGT TTC CCG ACT GGA AAG CGG	This study
lac-OE-rev	5′ - GCA TAT TCT CCT CGT GGG GTG TGA AAT TGT TAT CCG CTC AC	This study
cdiB-OE-for	5′ - GTG AGC GGA TAA CAA TTT CAC ACC CCA CGA GGA GAA TAT GC	This study
cdiB-Sac-rev	5′ - TTT GAG CTC GGA TTT GCT GCA GCC TGC	This study
lacI-Eco-for	5′ - TTT GAA TTC ACC ATC GAA TGG TGC AAA ACC	This study
CDI-up-Xho-rev	5′ - TTT CTC GAG TCA CTG CCC GCT TTC CAG TC	This study
CDI-up-Kpn-for	5′ - TTT GGT ACC GCG CGA TAA TTT CTT TCA GCC	This study

Reference

Ruhe, Z. C., A. B. Wallace, D. A. Low & C. S. Hayes, (2013) Receptor polymorphism restricts contact-dependent growth inhibition to members of the same species. *mBio* 4: e00480-00413.