

*Supplementary online information for:*

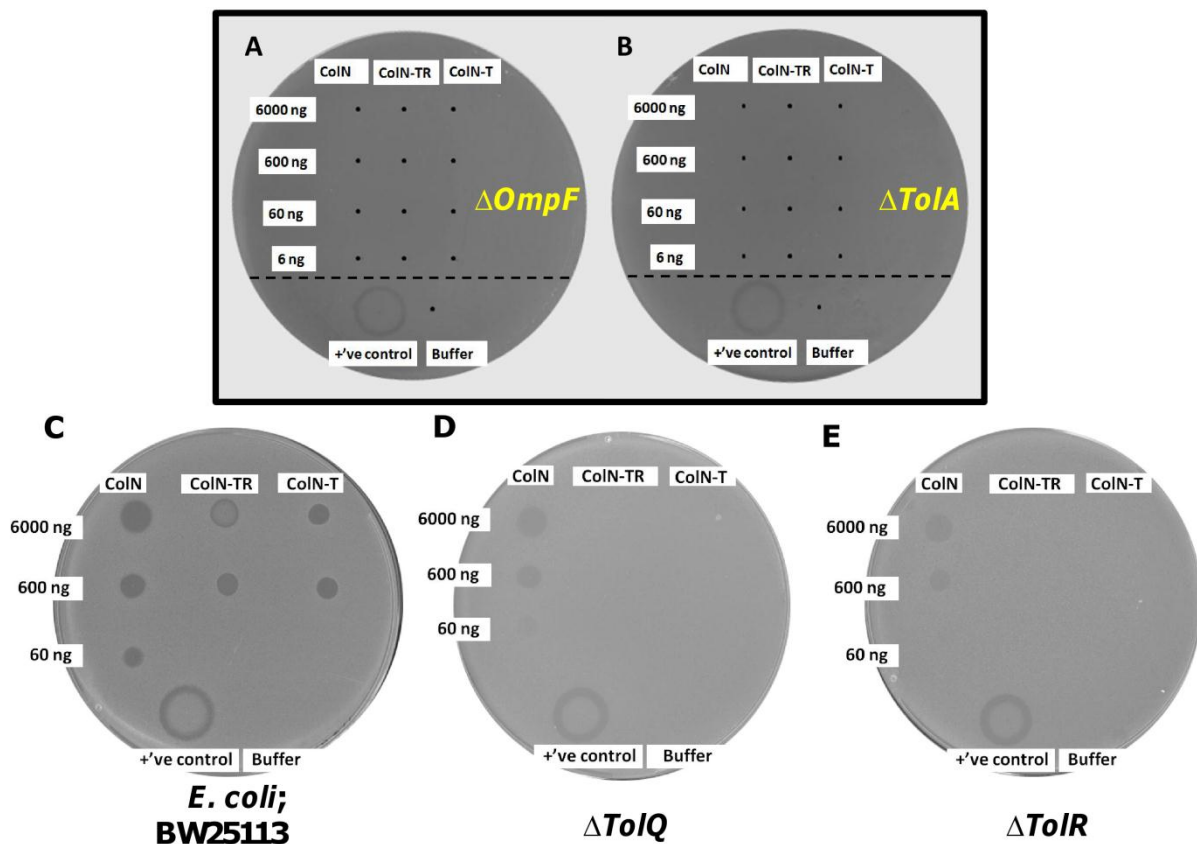
**The intrinsically unstructured translocation domain of colicin N kills *Escherichia coli*.**

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Supplementary figures.



**Figure S1 (A&B).** Spot test assay to demonstrate the OmpF, TolAQR dependency of ColN-TR and ColN-T killing. 2 $\mu$ l volumes corresponding to 6000 ng, 600 ng, 60 ng and 6 ng of ColN, ColN-TR and ColN-T were spotted onto a lawn of *E. coli*, either lacking **A**; OmpF (BZB1107) , **B**; TolA (JC207), **D**; TolQ (*E. coli*; BW25113 $\Delta tolQ$ ), **E**; TolR (*E. coli*; BW25113 $\Delta tolR$ ). The control strain *E. coli*; BW25113 is shown in (C) .As a negative control 2  $\mu$ l of the buffer in which the colicins were diluted (50 mM sodium phosphate, pH 7.5, 300 mM NaCl) was spotted. As a positive control 2 $\mu$ l (3000 ng) of ColIa (Cir and TonB dependent) was spotted.

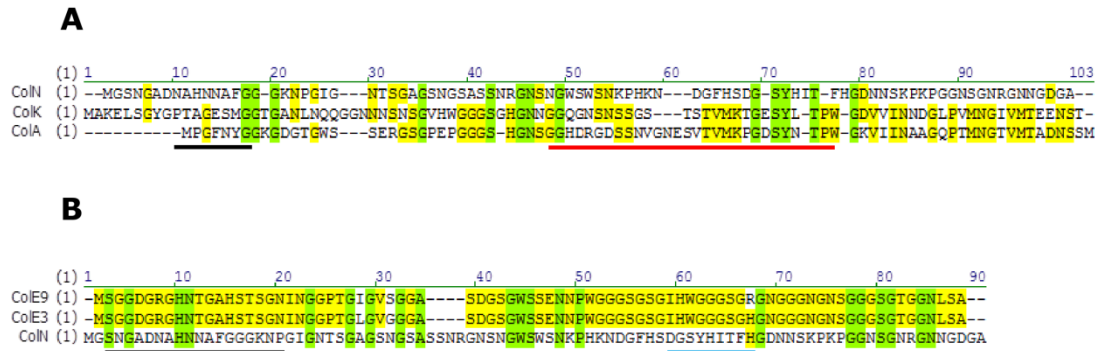
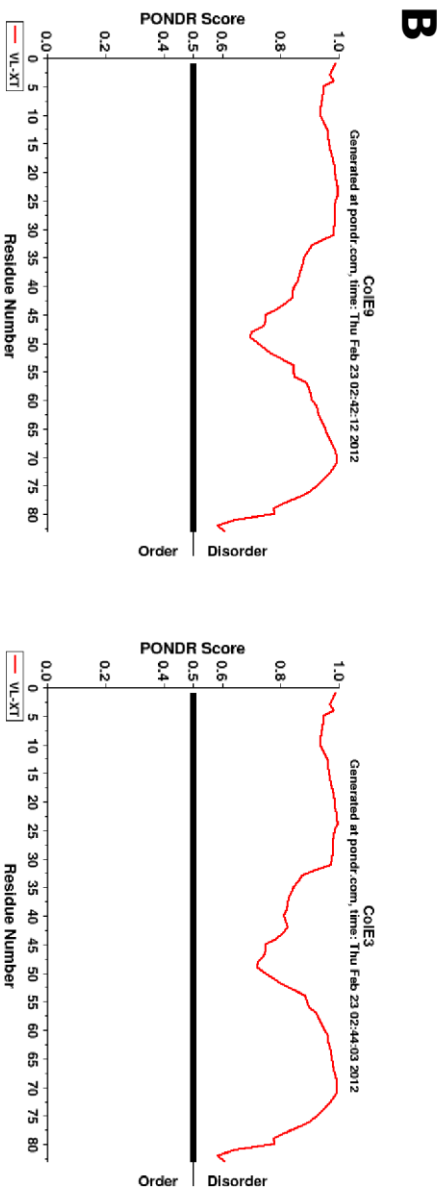
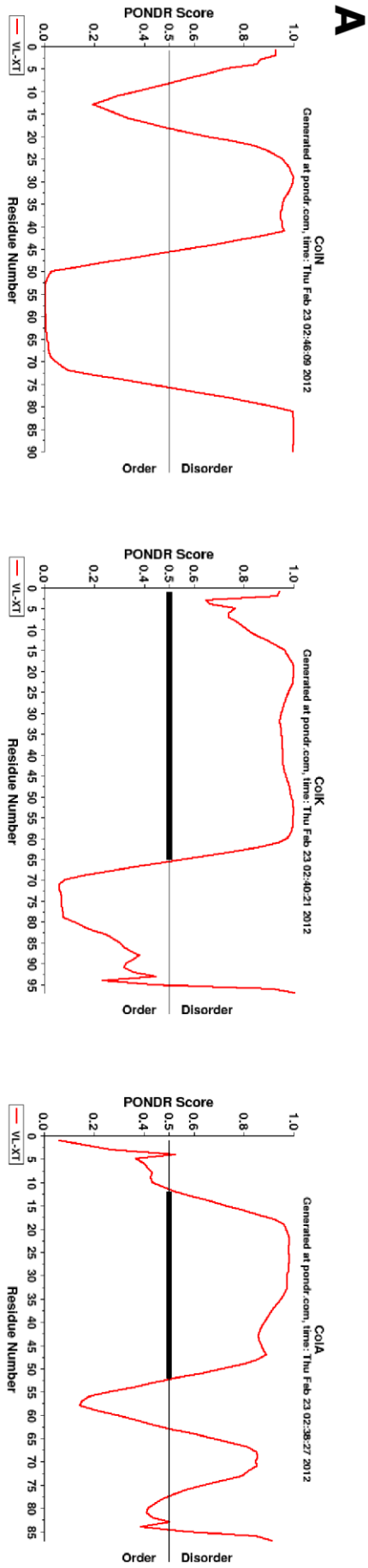
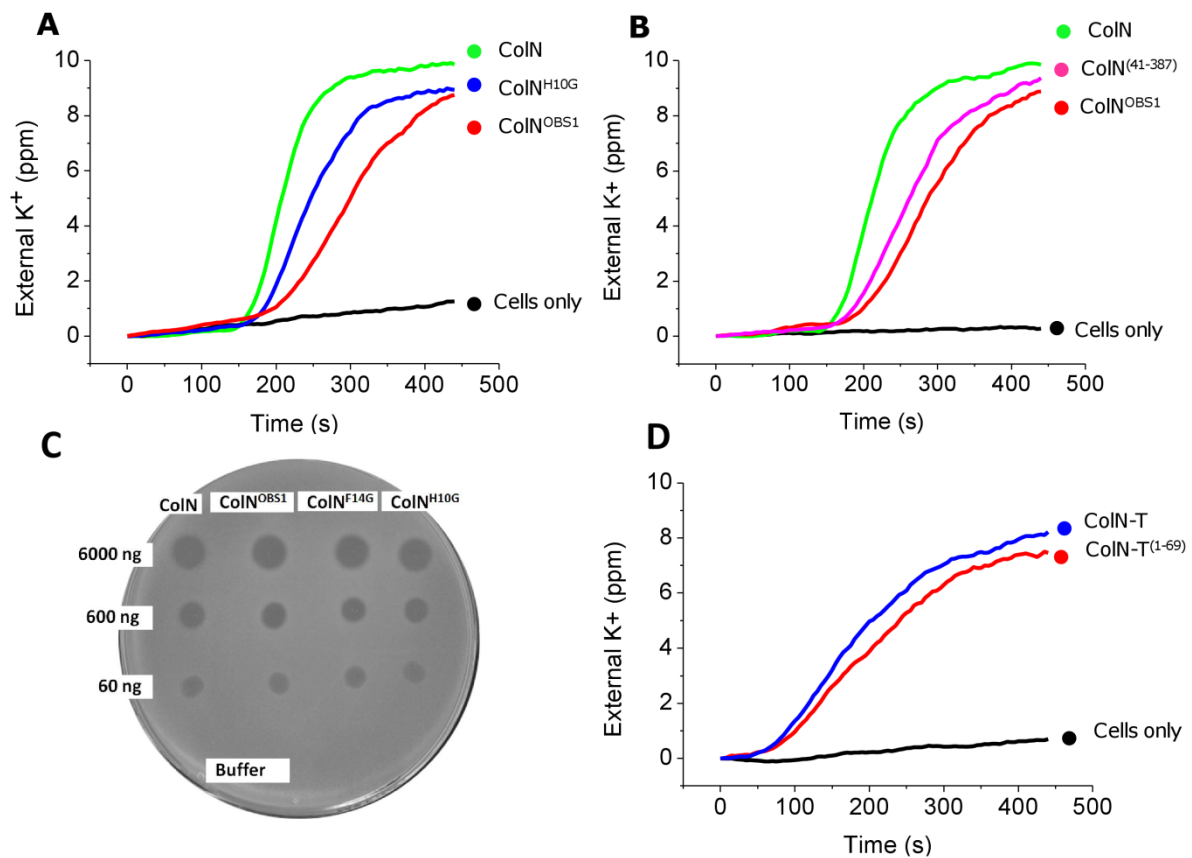


Figure S2

**Figure S2. Multiple alignments of colicin T-domains.** (A) Multiple alignment of the pore forming colicins ; colicin N (ColN), colicin K (ColK) and colicin A (ColA) which require OmpF for import and contain a TolA box. The minimal ColN OBS1 site is underlined in black and the ColN TolA box is underlined in red. (B). Multiple alignment of colicin N (ColN) with the enzymatic colicins E3 (ColE3) and E9 (ColE9) which require OmpF for import. The ColE9 OBS1 site is underlined in black and the OBS2 site in blue.

**Figure S3. PONDR plots of colicins which utilise OmpF for import. (A) PONDR plots of the pore forming colicins; colicin N (ColN), colicin K (ColK) and colicin A (ColA). (B) PONDR plots of the enzymatic colicins; colicin E9 (ColE9) and colicin E3 (ColE3).**





**Figure S4 A direct comparison of ColN OBS mutants.  $K^+$  efflux** (A) ColN<sup>OBS1</sup> (red) and ColN<sup>H10G</sup> (blue) were added at 600 mpc. ColN (green) added at 600 mpc is shown for reference, negative control (black). All colicins were added 120 seconds. (B) ColN<sup>OBS1</sup> (red) and ColN<sup>(41-387)</sup> (magenta) were added at 600 mpc. ColN (green) added at 600 mpc is shown for reference, negative to which no colicin is added (black). All colicins were added 120 seconds. (C) **Spot tests** 2 $\mu$ l volumes corresponding to 6000 ng, 600 ng, and 60 ng of ColN, ColN<sup>OBS1</sup>, ColN<sup>F14G</sup> and ColN<sup>H10G</sup> were spotted onto a lawn of BE3000. As a negative control 2  $\mu$ l of the buffer in which the colicins were diluted (50 mM sodium phosphate, pH 7.5, 300 mM NaCl) was spotted. (D)  **$K^+$  efflux of Colicin N-T<sup>(1-69)</sup>**. ColN-T (blue) and ColN-T<sup>(1-69)</sup> (red) were added at  $6 \times 10^5$  MPC at 0 seconds. Negative control to which no colicin is added to cells (black).

## Supplementary Table

**Table S1. MIC values of ColN OBS1 mutants.**

<b>Colicin Construct</b>	<b>MIC</b>
ColN <sup>OBS1</sup>	10 nM
ColN <sup>F14G</sup>	10 nM
ColN <sup>H10G</sup>	1 nM
ColN-TR <sup>OBS1</sup>	n/a