

Fig. S1. The FtsA, FtsZ and ZipA linkers all contain disordered regions. (A) PONDR VL-XT (pondr.com) prediction for FtsA. The inset region is indicated in the black box. The linker region is indicated in the shaded yellow box on the inset. (B) PONDR VL-XT prediction for FtsZ. The inset region is indicated in the black box, and the linker region is indicated in the shaded pink box on the inset. (C) PONDR VL-XT prediction for ZipA. The blue and green boxes correspond to the charged and P/Q-rich domains of the linker, respectively. Residues with a score higher than 0.5 are predicted to be intrinsically disordered.



Fig. S2. Multiple sequence alignment for ZipA proteins from different species. The Clustal Omega Multiple Sequence Alignment tool from the European Molecular Biology Laboratory- European Bioinformatics Institute (<u>https://www.ebi.ac.uk/Tools/msa/clustalo/;</u> see reference) was used to align the primary sequences of ZipA proteins from several different species, and MView (<u>https://www.ebi.ac.uk/Tools/msa/mview/</u>) was used to visualize the alignment. Sequences were collected from the NCBI Gene database. Sequences were collected from *Pseudomonas aeruginosa, Vibrio fischeri, Yersinia pestis, Klebsiella pneumoniae, Salmonella enterica, Escherichia coli,* and *Shigella dysenteriae*. Residues are colored by identity. Consensus sequences are shown below the species alignment.

Fig. S5. Cell length measurements of chromosomal ZipA linker mutants in various growth media. Cell lengths were measured in ImageJ from phase contrast images of cells grown to an OD between 0.3-0.4. A violin plot was assembled in Prism8 by GraphPad. Numbers above each violin correspond to the number of cells measured for each strain in each condition. The thick dashed line in each represents the average measured cell length, with the thinner dashed lines representing the 1st and 3rd quartiles.

Fig. S6. Overexpression of FtsA is less toxic in cells also overproducing

ZipA\DeltaP/Q, but not WT ZipA. WM 5337 (*zipA1*) cells cotransformed with pDSW210 derivatives expressing WT ZipA or ZipA Δ P/Q (labeled in blue) and either empty pKG116 vector or pKG116-FtsA (labeled in red) were serially-diluted 10-fold and spotted on LB plates with the indicated concentrations of IPTG (labeled in blue as it induces pDSW210 derivatives) and/or sodium salicylate (NaSal; labeled in red as it induces pKG116 derivatives) and incubated at 30°C or 42°C overnight. The asterisks indicate the rows with cells overexpressing both ZipA Δ P/Q and FtsA.

Table S1. Oligonucleotide primers used in this study.

Primer #	Sequence
2280	TCATGCCCCGGCTAACGCTCAGGAGCATGAGGCTGCTCGTTTATATTCCCCCAGAACATCA
2281	CGTTCATGATAATCACCGCTTCTTTGCGCTTCGGTTTATCATAGGAACTTCAAGATCCCCTTATTA
2196	TCAGTTGGCTCGTGGATC
2197	CTCTTTCCCATAGTGAAGC
2188	GCATCAGTTGGCTCGTGG
2189	ATGTGACTCTTTCCCATAGTG
2190	GATAAACCGAAGCGCAAAGAAG
2191	ACGAGCAGCCTCATGCTC
2192	CCGTCGCCGCAACACCAG
2193	GCTGGTCCAGAAACCATGTACC
2194	CAGCCGTTGCAGCAGCCA
2195	GACATCCTCGTCATAAGAATCGTCGTC
2163	GGCCATACCAAAAGCGAAGTGGCGGAAGAAGGCTCGTGGATCAAGCGA
2164	GCGGCTTTCGTTTTTGCCCACCAGCACCGCATAGTGAAGCAATCCCACCG
2159	GGCTCGTGGATCAAGCGA
2160	ATAGTGAAGCAATCCCACCG
265	TGCAGGATTTGCGTCTGATA
2364	CAGGAAAATTCTGCGTATTTTAC
2365	CGCGCAGTTCGCGCATCAG
2366	TATATATCTAGATCAGGCGTTGGCGTCTTTGAC
1157	CCGACATCATAACGGTTCTGGCA
1158	GCCATCTAATTCAACAAGAACTG
1225	TGCCGCCAGGCAAATTCTGTTTTATCAGAC

Description ZipA ΔP/Q ccdB:kan forward ZipA ΔP/Q ccdB:kan reverse FtsA ∆5-19 forward FtsA ∆5-19 reverse FtsA ∆5-15 forward FtsA ∆5-15 reverse ZipA ∆PQ forward $ZipA \Delta PQ$ reverse $ZipA \Delta + /-$ forward ZipA∆+/- reverse ZipA $\Delta^{1/2}$ forward $ZipA\,\Delta ^{1\!\!/_{\!\!2}}\,reverse$ FtsA scrambled linker insertion forward FtsA scrambled linker insertion reverse FtsA ∆1-20 forward FtsA∆1-20 reverse Internal ZipA forward Forward primer for sequencing ZipA in chromosome Reverse primer for sequencing ZipA in chromosome Internal ZipA reverse pDSW210 vector forward primer pDSW210 vector reverse primer for ZipA-GFP fusions pDSW210 vector reverse primer for FtsA linker mutants