

Supporting Information

Supporting Figure Legends

Figure S1. (A) Quantitative immunoblot of lysates from wild type and *ezrA* TM mutant cells. All constructs were expressed from the native promoter with the exception of *ezrAΔTM*, which was expressed from the IPTG inducible *Pspachy* promoter. FtsZ loading control on bottom. Primary rabbit antisera raised against EzrA or FtsZ was detected using secondary anti-rabbit serum conjugated to horse radish peroxidase. (B) Immunoblot of membrane fractionation of wild type and *ezrA* TM mutant lysates. As expected, wild type EzrA and TM helix chimeras are concentrated in the membrane fraction. The TM-less mutant is only in the cytoplasmic fraction.

Figure S2. (A) Quantitative immunoblot of coiled-coil deletion constructs. FtsZ loading control on bottom. Note faint TM-QNR-GFP band on far right, consistent with degradation. (B) (Top three panels) Representative immunoblots of membrane fractionations from coiled-coil deletion strains. Three independent experiments are presented. Degradation over the course of the two-day assay, the latter steps of which were performed in the absence of protease inhibitors, led to variation in protein levels between experiments. Molecular weight marker is visible on far left of all three blots (whitish band). (Bottom) Soluble control (FtsZ). (C) Immunoblot of soluble fractions from coiled-coil deletion strains probed with anti-GFP sera (top) or anti-FtsZ sera (bottom). No GFP was visible in soluble fractions, consistent with membrane retention of all CC deletion mutants. Primary rabbit antibody against GFP (Genscript) or FtsZ was detected using secondary anti-rabbit serum conjugated to HRP.

Figure S3. (A) Micrographs of GFP fusions to wild type EzrA, the full length EzrA(R510D) mutant, and an EzrA deletion mutant [*ezrAΔ(31-499)*] that includes EzrA's native transmembrane helix and QNR patch but is missing all four coiled-coils. Note absence of medial localization in both the *ezrA(R510D)* and *ezrAΔ(31-499)* images. (B) FtsZ localization by immunofluorescence microscopy. Note the presence of polar FtsZ rings in the *ezrAΔ(31-499)* images. (A and B). Thick arrows indicate medial EzrA and FtsZ localization. Thin arrows indicate EzrA localization at septa. Arrowheads indicate polar FtsZ rings. Exposure times are equivalent for each fluorophore. Bars = 5 μ m. (C) Consistent with loss of medial localization, the *ezrAΔ(31-499)* allele is equivalent to an *ezrA* null with regard to its ability to suppress the lethality associated with overexpression of the MinCD inhibitor or the heat sensitivity of the *ftsZts* allele. Bars equal standard deviation from three repeated experiments.

Supporting References

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Table S1. Bacterial Strains Used in this Study

<i>B. subtilis</i> strains	Genotype	Reference
JH642	<i>B. subtilis</i> trpC2 pheA1	[1]
PL874	JH642 <i>ftsZ::ftsZ-gfp</i> spc	[2]
PL642	JH642 <i>ftsZ::ftsZ-gfp</i> cat	[2]
PL1138	JH642 <i>amyE::P_{spachy}-minCD</i> cat	[3]
PL1145	JH642 <i>thrC::P_{spachy}-minCD</i> MLS	[4]
PL982	JH642 <i>amyE::P_{spac}-ftsZ-gfp</i> cat	This work
PL851	PL847 <i>ezrA-gfp</i> , <i>P_{spac}-ftsZ</i>	[2]
PL1776	JH642 <i>ezrA::ezrA(R510D)-gfp</i> spc	[5]
PL2104	JH642 <i>thrC::P_{spac}-ftsZ-gfp</i> MLS	This work
PL3040	JH642 <i>amyE::P_{spachy}-ezrAΔTM</i> cat	This work
PL3054	JH642 <i>amyE::P_{spachy}-ezrAΔTM</i> cat, <i>ftsZ::ftsZ-gfp</i> spc	This work
PL2719	JH642 <i>ezrA</i> TM:: <i>cccA</i> TM spc	This work
PL2725	JH642 <i>ezrA</i> TM:: <i>cccA</i> TM spc, <i>amyE::P_{spac}-ftsZ-gfp</i> cat	This work

PL2723	JH642 <i>ezrA</i> TM:: <i>cccA</i> TM spc, <i>amyE</i> ::P _{<i>spachy</i>} - <i>minCD</i> cat	This work
PL2721	JH642 <i>ezrA</i> TM:: <i>cccA</i> TM spc, <i>ftsZ</i> :: <i>ftsZ-gfp</i> cat	This work
PL2741	JH642 <i>ezrA</i> TM:: <i>ftsK</i> TM spc	This work
PL2745	JH642 <i>ezrA</i> TM:: <i>ftsK</i> TM spc, <i>amyE</i> ::P _{<i>spac</i>} - <i>ftsZ-gfp</i> cat	This work
PL2743	JH642 <i>ezrA</i> TM:: <i>ftsK</i> TM spc, <i>amyE</i> ::P _{<i>spachy</i>} - <i>minCD</i> cat	This work
PL2744	JH642 <i>ezrA</i> TM:: <i>ftsK</i> TM spc, <i>ftsZ</i> :: <i>ftsZ-gfp</i> cat	This work
PL2748	JH642 <i>ezrA</i> TM:: <i>sdhA</i> TM spc	This work
PL2751	JH642 <i>ezrA</i> TM:: <i>sdhA</i> TM spc, <i>amyE</i> ::P _{<i>spac</i>} - <i>ftsZ-gfp</i> cat	This work
PL2749	JH642 <i>ezrA</i> TM:: <i>sdhA</i> TM spc, <i>amyE</i> ::P _{<i>spachy</i>} - <i>minCD</i> cat	This work
PL2750	JH642 <i>ezrA</i> TM:: <i>sdhA</i> TM spc, <i>ftsZ</i> :: <i>ftsZ-gfp</i> cat	This work
PL1548	JH642 <i>ezrA</i> TM:: <i>zipA</i> TM spc	This work
PL2692	JH642 <i>ezrA</i> TM:: <i>zipA</i> TM spc, <i>amyE</i> ::P _{<i>spac</i>} - <i>ftsZ-gfp</i> cat	This work
PL2701	JH642 <i>ezrA</i> TM:: <i>zipA</i> TM spc, <i>amyE</i> ::P _{<i>spachy</i>} - <i>minCD</i> cat	This work
PL2647	JH642 <i>ezrA</i> TM:: <i>zipA</i> TM spc, <i>ftsZ</i> :: <i>ftsZ-gfp</i> cat	This work
PL2694	JH642 <i>ezrA</i> TM:: <i>zipA</i> TM spc, <i>thrC</i> ::P _{<i>spac</i>} - <i>ftsZ-gfp</i> MLS	This work

PL3042	JH642 <i>ezrA::ezrA-gfp</i> spc, <i>amyE::P_{spachy}-minCD</i> cat	This work
PL3114	JH642 <i>ezrA::ezrA-gfp</i> spc, <i>ftsZ::ftsZ-gfp</i> cat	This work
PL2734	JH642 <i>ezrA::ezrA-cfp</i> cat, <i>thrC::P_{spac}-ftsZ-gfp</i> MLS	This work
PL2752	JH642 <i>ezrA::kan kan</i> , <i>amyE::P_{spac}-ftsZ-gfp</i> cat	This work
PL2533	JH642 <i>ezrA::kan kan</i> , <i>amyE::P_{spachy}-minCD</i> cat	This work
PL2642	JH642 <i>ezrA::kan kan</i> , <i>ftsZ::ftsZ-gfp</i> cat	This work
PL2543	JH642 <i>ezrA::kan kan</i> , <i>thrC::P_{spachy}-minCD</i> MLS	This work
PL2982	JH642 <i>ezrA::P_{spachy}-ezrAΔTM</i> cat, <i>amyE::P_{spachy}-ezrA-spc</i> spc	This work
PL2700	JH642 <i>ezrA::P_{spachy}-ezrAΔTM</i> cat, <i>ftsZ::ftsZ-gfp</i> spc	This work
PL2690	JH642 <i>ezrA::P_{spachy}-ezrAΔTM</i> cat, <i>thrC::P_{spac}-ftsZ-gfp</i> MLS	This work
PL2681	JH642 <i>ezrA::P_{spachy}-ezrAΔTM</i> cat, <i>thrC::P_{spac}-minCD</i> MLS	This work
PL2611	JH642 <i>ezrAΔ(31-353)</i> spc	This work
PL2715	JH642 <i>ezrAΔ(31-353)-gfp</i> spc cat	This work
PL3051	JH642 <i>ezrAΔ(31-353)-gfp</i> , <i>thrC::P_{spachy}-minCD</i>	This work
PL2686	JH642 <i>ezrAΔ(31-353)</i> , <i>amyE::P_{spac}-ftsZ-gfp</i>	This work

PL2682	JH642 <i>ezrAΔ(31-353)</i> , <i>amyE::P_{spachy}-minCD</i>	This work
PL2613	JH642 <i>ezrAΔ(31-353)</i> , <i>ftsZ::ftsZ-gfp</i>	This work
PL2688	JH642 <i>ezrAΔ(31-353)</i> , <i>thrC::P_{spac}-ftsZ-gfp</i>	This work
PL2705	JH642 <i>ezrAΔ(31-499)</i>	This work
PL2713	JH642 <i>ezrAΔ(31-499)-gfp</i>	This work
PL3052	JH642 <i>ezrAΔ(31-499)-gfp</i> , <i>thrC::P_{spachy}-minCD</i>	This work
PL2711	JH642 <i>ezrAΔ(31-499)</i> , <i>amyE::P_{spac}-ftsZ-gfp</i>	This work
PL2709	JH642 <i>ezrAΔ(31-499)</i> , <i>amyE::P_{spachy}-minCD</i>	This work
PL2707	JH642 <i>ezrAΔ(31-499)</i> , <i>ftsZ::ftsZ-gfp</i>	This work
PL3017	JH642 <i>ezrAΔ(191-353)</i>	This work
PL3019	JH642 <i>ezrAΔ(191-353)-gfp</i>	This work
PL3053	JH642 <i>ezrAΔ(191-353)-gfp</i> , <i>thrC::P_{spachy}-minCD</i>	This work
PL3119	JH642 <i>ezrAΔ(191-353)</i> , <i>ftsZ::ftsZ-gfp</i>	This work
PL3045	JH642 <i>ezrAΔ(376-434)-gfp</i> , <i>amyE::P_{spachy}-minCD</i>	This work
PL3116	JH642 <i>ezrAΔ(376-434)-gfp</i> , <i>ftsZ::ftsZ-gfp</i>	This work

PL3023	JH642 <i>ezrAΔ(377-493)-gfp</i>	This work
PL3044	JH642 <i>ezrAΔ(377-493)-gfp, amyE::P_{spachy}-minCD</i>	This work
PL3120	JH642 <i>ezrAΔ(377-493)-gfp, ftsZ-gfp</i>	This work
PL2636	JH642 <i>ezrAΔ(377-562)</i>	This work
PL872	JH642 <i>ezrAΔ(377-562)-gfp</i>	This work
PL3043	JH642 <i>ezrAΔ(377-562)-gfp, amyE::P_{spachy}-minCD</i>	This work
PL3115	JH642 <i>ezrAΔ(377-562)-gfp, ftsZ-gfp</i>	This work
PL2696	JH642 <i>ezrAΔ(377-562), amyE::P_{spac}-ftsZ-gfp</i>	This work
PL2684	JH642 <i>ezrAΔ(377-562), amyE::P_{spachy}-minCD</i>	This work
PL2614	JH642 <i>ezrAΔ(377-562), ftsZ::ftsZ-gfp</i>	This work
PL2652	JH642 <i>ezrAΔ(377-562), ftsZ::ftsZ-gfp</i>	This work
PL2698	JH642 <i>ezrAΔ(377-562), thrC::P_{spac}-ftsZ-gfp</i>	This work
PL3046	JH642 <i>ezrAΔ(468-504)-gfp, amyE::P_{spachy}-minCD</i>	This work
PL3117	JH642 <i>ezrAΔ(468-504)-gfp, ftsZ-gfp</i>	This work
<i>E. coli</i> strains	Genotype	Reference

AG1111	DZR200=MC1061 F' <i>lacIQ lacZ</i> M15 Tn10 tet	[6]
Top10	F- <i>mcrA</i> Δ(mrr-hsdRMS-mcrBC) φ80lacZ ΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(StrR) endA1 λ-	Invitrogen
LMG194	F- ΔlacX74 galE thi rpsL ΔphoA (Pvu II) Δara714 leu::Tn10	Invitrogen

Table S2. Plasmids used in this study

Plasmid	Genotype	Reference
pBAD	Overexpression vector for N-terminal His thioredoxin fusion	Life Technologies
pUS19	Integrational plasmid for spectinomycin resistance for <i>B. subtilis</i>	New England Biolabs
pPL82	P _{spachy} integration vector	[7]
pJL74	Shuttle plasmid replicating in both <i>E. coli</i> and <i>B. subtilis</i>	[8]
pRS3	QuickChange Site Mutagenesis	Stratagene
pPL65	pUS19: ~900-bp 3' ezrA fragment fused to gfp	[2]
pPL3090	pBAD/Thio-ezraΔ(1-203)-His	This work
pPL2984	pBAD/Thio-ezraΔ(191-353)-His	This work
pPL2829	pBAD/Thio-ezraΔ(376-434)-His	This work
pPL2883	pBAD/Thio-ezraΔ(468-504)-His	This work
pPL3094	pBAD/Thio-ezraΔ(468-562)-His	This work
pPL2998	pBAD/Thio-ezraΔ(501-511)-His	This work
pPL2780	pBAD/Thio-ezra(494-562)-His (Thio-QNR-His)	This work
pPL2784	pBAD/Thio-QNR(R510D)-His	This work

pPL2609	pJL74: <i>ezraΔ(31-353)</i>	This work
pPL2986	pJL74: <i>ezraΔ(191-353)</i>	This work
pPL2703	pJL74: <i>ezraΔ(31-499)</i>	This work
pPL2607	pJL74: <i>ezraΔ(377-562)</i>	This work
pPL1232	pJL74:~900-bp 3' <i>ezrA</i> fragment fused to <i>gfp</i>	This work
pDH26	pUS19: <i>ezraΔ(376-434)-gfp</i>	This work
pDH27	pUS19: <i>ezraΔ(468-504)-gfp</i>	This work
pPL69	pUS19: <i>ezraΔ(377-562)-gfp</i>	This work
pPAP1	pJL74: <i>ezraTM::zipATM</i>	This work
pPL2717	pJL74: <i>ezraTM::cccATM</i>	This work
pPL2739	pJL74: <i>ezraTM::ftsKTM</i>	This work
pPL2746	pJL74: <i>ezraTM::sdhATM</i>	This work
pPL3038	pPL82:P _{<i>spachy</i>} - <i>ezraΔTM</i>	This work

Table S3. Primers used in this study

	Cloning <i>ezrA</i> full length to TOPO vector
PLO 99	ATCTACGCCGAAATCGACCGGCTG
PLO 141	AGCGGATATGTCAGCTTGATTTTTC
	Cloning N-<i>ezrA</i> to TOPO vector
PLO 99	ATCTACGCCGAAATCGACCGGCTG
PLO 147	GGCGACATGCTCTGCATCAAGC
	Cloning C-<i>ezrA</i> to TOPO vector
PLO 102	AGGCTGACAGCCGGTGAGCTCGGC
PLO 141	AGCGGATATGTCAGCTTGATTTTTC
	<i>ezrA</i> deletion
PLO <i>ezrAF1</i>	GATAGGAAGAGTCTGTGC

PLO <i>ezrAR1</i> -BamHI	GATC GGATCC ACAAACTCCATAATGAGC
PLO <i>ezrA</i> - F2-HindIII	GATC AAGCTT TCCGCTTAGATAATCACG
PLO <i>ezrA</i> -R2	CGGCCTTGTACACATCTTG
	<i>ezrA</i> N-terminus deletion
PLO 990 <i>ezrA</i> -F1-Sall	GATC GTCGAC GGCATAGATAGGAAGAGTCTGTGC
PLO 991 <i>ezrA</i> -R1-EcoRI	GATC GAATTCACTGACAAACTCCATAATGA
PLO 992 <i>ezrA</i> -F3-Spel-2	GATC ACTAGT AAAGCGCCTTGATGAAATTG
PLO 993 <i>ezrA</i> -R3-Sacl	GATC GAGCTCTTACACTGCAGGGCTTTTT
	<i>ezrA</i> C-terminus deletion
PLO 994 <i>ezrA</i> -F2-Sall	GATC GTCGAC CCTGAGAGATTAAGGTAAACAGCA
PLO 995 <i>ezrA</i> -R2-EcoRI	GATC GAATTCTGAGTAGGCGACATGCTCTG

PLO 1019 <i>ezrA</i> -R2- EcoRI-stop	GATC GAATTCTGACTAGGGCGACATGCTCTG
PLO 996 <i>ezrA</i> -F4-Spel	GATC ACTAGT GCTGACATATCCGCTTAGATAATCA
PLO 997 <i>ezrA</i> -R4-Sacl	GATC GAGCTCTACTCGGAGCAGCGATTACA
	<i>ezrA</i> promoter and TM
PLO 1017 promoter+TM- F-BamHI	GATC GGATCC CGCTTTGCATTGATGTCAC
PLO 1018 promoter+TM- R-Spel	GATC ACTAGT TCGATTCGGCGTAGATT
	<i>ezrA</i> CC2 deletion
del498-986	TTGAAGAACAGAAGGCCGGAAAAAGCGCCTTGATG
del498-986- antisense	CATCAAGGCGCTTTCCGCCTCTGTTCTTCAA
	<i>ezrA</i> CC3 deletion
PLO 419	CTTGATGCAGAGCATGTCACTAGAACATTCCAGGC
PLO 420	GCCTGGAATGTTGCTAGTGACATGCTGTGCATCAAG

	ezrA CC4 deletion
PLO 421	CAACTAACGAACCTCCAATTCAAGTCGGAAACCGG
PLO 422	CCGGTTTCCGAACTGAATTGGAAGTCGTTAGTTG
	ezrA CC1 & CC2 deletion
F-ezrA-Swal	GATC ATTTAAATCACCCCTGACAGATTAAGGTAAAC
R-ezrA-Ndel	GATC CATATGTTAACGGATATGTCAGCTTG
	ezrA CC3 & CC4 deletion
PLO 1059 ezrA-F5-PstI	GATC CTGCAG CTT GTC GAG CAG GTC ATC CT
PLO 1060 ezrA-R5-PstI	GATC CTGCAG TGA TTA TCT AAG CGG ATA TGT CAG C
PLO 1061 ezrA-R5-XbaI	GATC TCTAGA TGA TTA TCT AAG CGG ATA TGT CAG C
	ezrA TM::zipA TM swap

PLO 521 P1 w/EcoRI	GATCGAATTCTCGCTTGCATTGATGTCACCCCATGAAAAAA ATAG
PLO 522 P2	CAGACGCAAATCCTGCATCATAATGAGCCCCCTGCTGTTAC CTTAATCTG
PLO 523 P3	GTAAACAGCAAGGGGGCTCATTATGATGCAGGATTGCGTCT GATATT
PLO 524 P4	CGGCGTAGATTTTTCTGCTGGTCCAGAAACCATGTACCAAG TAAAGC
PLO 525 P5	GTACATGGTTCTGGACCAGCAGGAAAAAAATCTACGCCGAA ATCGACC
PLO 526 P6 w/ Xmal	GATC CCCGGG CTAAGCGGATATGTCAGCTTGATTTTCAACTG
PLO 527 P7 w/HindIII	GATC AAGCTT AACCTCGGCATGTGGCTGTGAC
	ezrA TM::cccA TM swap
PLO 521 P1 w/EcoRI	GATCGAATTCTCGCTTGCATTGATGTCACCCCATGAAAAAA ATAG
PLO 1042 P2 ezrA Prom- c550-R	AATAAGCGGGTTCCATTCATAATGAGCCCCCTGCTGTTAC CTTAATCTG
PLO 1043 P3 c550 TM-F	GTAAACAGCAAGGGGGCTCATTATGAAATGGAACCCGCTTATT CCATTTTG

PLO 1044 p4 c550 TM-R	CGGCGTAGATTTTTCTAAGTCCTTTACTGATAAAAAGAAA GTTAGACC
PLO 1045 P5 N-ezrA-c550-F	TTTTTATCAGTAAAGGACTTAGGAAAAAAAATCTACGCCGAA ATCGACC
PLO 527 P7 w/HindIII	GATC AAGCTT AACCTCGGCATGTGGGCTGTGAC
	ezrA TM::ftsK TM swap
PLO 521 P1 w/EcoRI	GATCGAATTCTCGCTTGCATTGATGTCACCCCATGAAAAAA ATAG
PLO 1081 P2- <i>ftsK</i>	AGAGCAGCGCAATAGTTCCCATAATGAGCCCCCTGCTGTTA CCTTAATCTG
PLO 1082 P3- <i>ftsK</i>	TAAACAGCAAGGGGGCTCATTATGGAACTATTGCGCTGCTC TGCCTT
PLO 1083 P4- <i>ftsK</i>	TCGGCGTAGATTTTTCTGAGTTTCAGCAATGGTCACCC ATGACCAACC
PLO 1084 P5- <i>ftsK</i>	GTGACCATTGCTGAAAAACTCAGGAAAAAAAATCTACGCCGAAA TCGACC
PLO 527 P7 w/HindIII	GATC AAGCTT AACCTCGGCATGTGGGCTGTGAC
	ezrA TM::sdhA TM swap

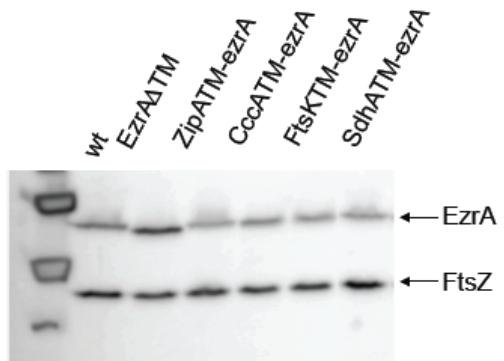
PLO 521 P1 w/EcoRI	GATCGAATTCTCGCTTGCATTGATGTCACCCCATGAAAAAA ATAG
PLO 1085 P2- <i>sdhA</i>	GATAATGCTTGATTGACTCATAATGAGCCCCCTGCTGTTAC CTTAATCTG
PLO 1086 P3- <i>sdhA</i>	TAAACAGCAAGGGGGCTCATTATGAGTCAATCAAGCATTATCG TAGTCGGCG
PLO 1087 P4- <i>sdhA</i>	TCGGCGTAGATTTTTCTCGCCATTCTGATTCCGCTGCTT TAATTGTC
PLO 1088 P5- <i>sdhA</i>	GCAGCGGAATCAGGAATGGCGAGGAAAAAAATCTACGCCGAA ATCGACC
PLO 527 P7 w/HindIII	GATC AAGCTT AACTTCGGCATGTGGCTGTGAC
	ezrA TM deletion
PLO 1113 pJQ43-F1	TGCCACCTGACGTCTAAGAA
PLO 1114 pJQ43-F2	AAGTTGGCCGCAGTGTATC
PLO 1115 pJQ43-F2- Spel	GATC ACTAGT AAGTTGGCCGCAGTGTATC
PLO 669	GATCGGATCCCTAACGGATATGTCAGCTTG
	cloning ezrA(QNR) to TOPO vector
PLO 1116 F5- ezrA	CTTGTGAGCAGGTACCT

PLO 141

AGCGGATATGTCAGCTTGATTTC

Figure S1

A



B

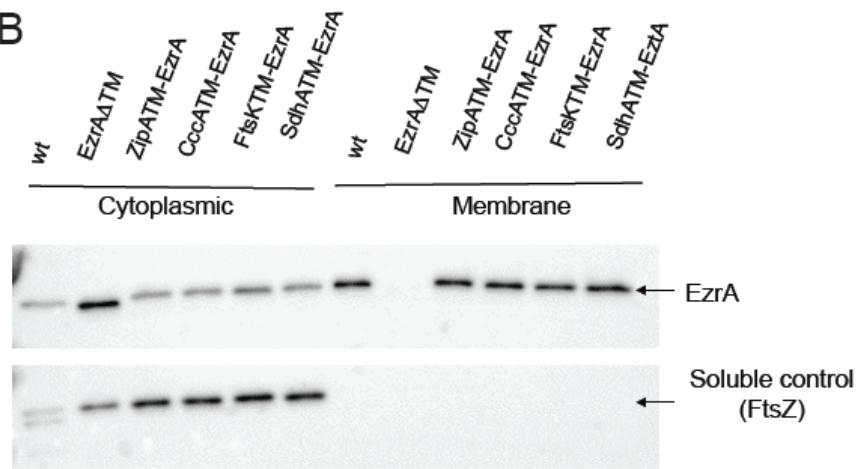


Figure S2

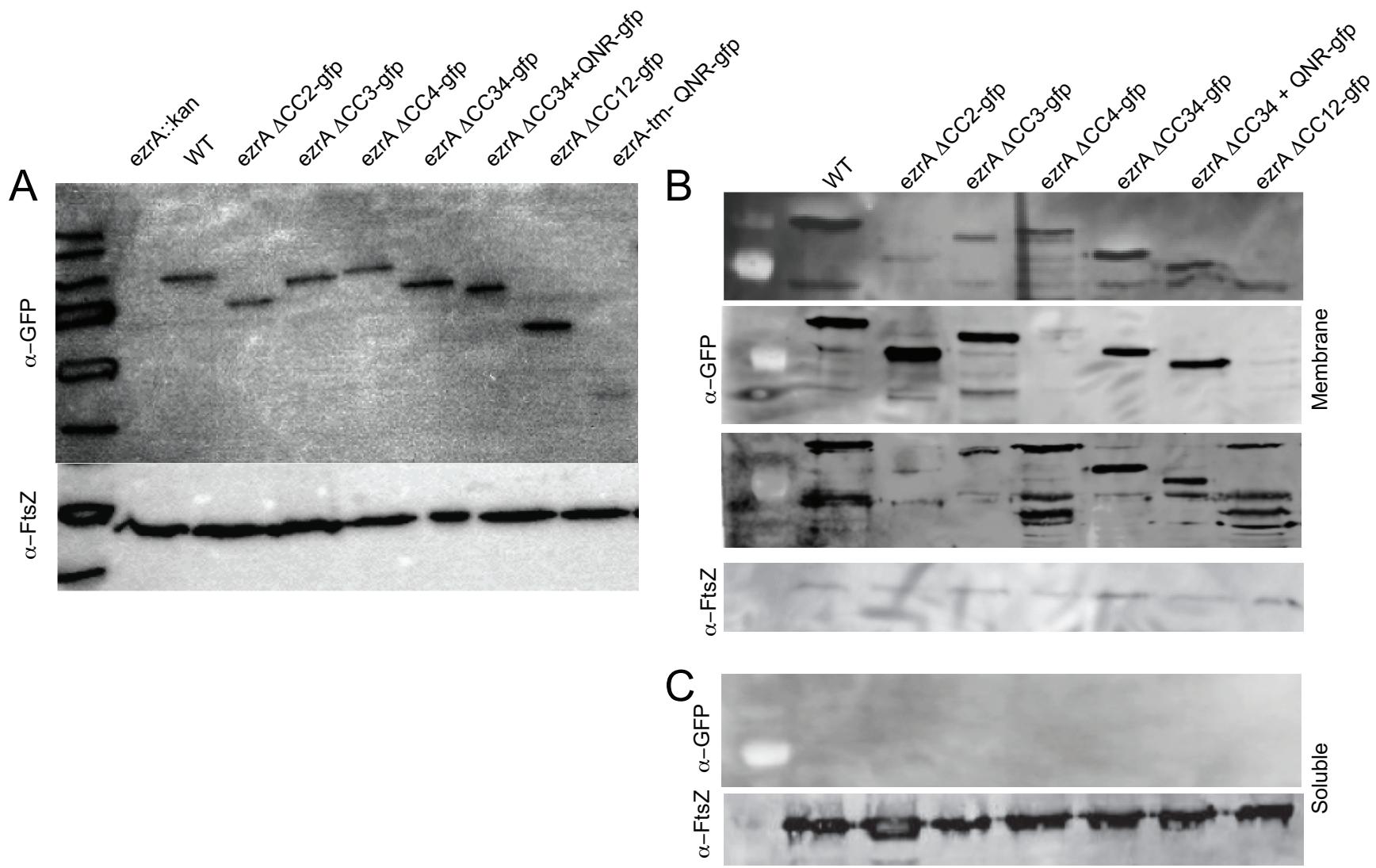


Figure S3

