Table S1. Strain, plasmid, and oligonucleotide list

	Description	Reference
Strains		
P90C	F- *ara* Δ(*lac-pro*)$_{XII}$ *thi*	(1)
Δ*dinB*	As P90C but with Δ*dinB*::Kan	(2)
RWB1581	As P90C but *dnaE915* zae-502::Tn10 zae::Tn10dcam from SMR6114 (3) replacing *dnaE*$^+$	This work
RWB2028	As RWB1581 but with Δ*dinB*::frt	This work.
RWB2031	As Δ*dinB* but with Δ*attB*::*sulAp*-GFP and *gal76*::Tn10 from SS1465 (4)	This work
RWB2132	As RWB2028 but with Δ*attB*::*sulAp*-GFP and *gal76*::Tn10 from SS1465 (4)	This work
RWB2370	As RWB2028 but with (ΔTLS) Δ*polB*::Kan and Δ*umuDC*::cat	This work
RWB2436	As RWB1581 but with *dinB(D103N)* (5) replacing *dinB*$^+$	This work
RWB2184	As RWB2132 but with Δ*recA*::Kan	This work
RWB3374	As RWB2436 but with Δ*umuDC*::cat.	This work
RWB3404	As RWB3374 but with Δ*dinB*::Kan	This work
RWB3401	As RWB2028 but with Δ*polB*::Kan	This work
MG1655	Wild type *Escherichia coli*	(6)
RWB169	As MG1655 but with *dnaE915* and Δ*dinB*::Kan alleles	This work
BL21-AI Δ*dinB*	BL21-AI Δ*dinB*::Kan	(7)
Plasmids		
pWSK29	pSC101 replicon with pBluescript II SK$^+$ multiple cloning site, Amp^R	(8)
pYG768	pWSK29 with *dinB*$^+$ under its native promoter	(9)
pYG768(D103N)	As pYG768 but with *dinB(D103N)*	(10)
pYG768Δβ	As pYG768 but with *dinB*$^+$Δβ	Lab Stock
pYG768(D103N)Δβ	As pYG768 but with *dinB(D103N)*Δβ	(2)
pYG768(V288G)	As pYG768 but with *dinB(V288G)*	This work
pYG768(F292Y)	As pYG768 but with *dinB(F292Y)*	This work
pYG768(V7G)	As pYG768 but with *dinB(V7G)*	This work
pBAD18(*dinB*$^+$)	*dinB*$^+$ under the ara promoter inserted with KpnI and XbaI restriction sites into pBAD18 (11)	This work
pBAD18(*dinB(D103N)*)	As pBAD18(*dinB*$^+$) but with *dinB(D103N)*	This work
pBAD18(*dinB(F292Y)*)	As pBAD18(*dinB*$^+$) but with *dinB(F292Y)*	This work
pBAD18(*dinB(V7G)*)	As pBAD18(*dinB*$^+$) but with *dinB(V7G)*	This work
pET11T(*dinB*$^+$)	pETT11T (12) overexpression vector with untagged *dinB*$^+$	(13)
pET11T(*dinB(D103N)*)	As pET11T(*dinB*$^+$) but with *dinB(D103N)*	(7)
pET11T(*dinB(F292Y)*)	As pET11T(*dinB*$^+$) but with *dinB(F292Y)*	This work
Oligonucleotides		
D103N mutagenesis forward primer	GAACCGTTGTCACTGAATGAGGCTTATC	(2)
D103N mutagenesis reverse primer	CAGTGACAACGGTTCAATGCGCGAG	(2)

V288G mutagenesis forward primer	CTGATTGCTCGCCAGGGGGGGAAATTAAAG	This work
V288G mutagenesis reverse primer	CCCCTGGCGAGCAATCAGTAAATCAGGTTT	This work
F292Y mutagenesis forward primer	CCAGGGGGTGAAATTAAAGTACGACGATTTTC	This work
F292Y mutagenesis reverse primer	ACTTTAATTTCACCCCCTGGCGAGCAATCAG	This work
V7G mutagenesis forward primer	GCGTAAAATCATTCATGGGGATATGGAC	This work
V7G mutagenesis reverse primer	CACATGAATGATTTTACGCATTGCTCACCTC	This work
*dinB-Kpn*I primer for adding *Kpn*I restriction site upstream *dinB*	GAGTAAGGTACCGTTGAGAGGTGAGCAATGCG	This work
*dinB-Xba*I primer for adding *Xba*I restriction site downstream *dinB*	GCCTCCTCTAGACATCATAATCCCAGCACCAGTTG	This work
Undamaged control template for primer extension assay	GCT CGT CAG ACG ATT TAG AGT CTG CAG TG	(7)
Lesion containing template for primer extension assay	GCTCGTCAGACG/3-deaza-3-methylA/TTTAGAGTCTGCAGTG	(7)
Fluorescently labeled primer for standing start primer extension	/HEX/CACTGCAGACTCTAAA	(7)

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Supplemental Figure Legends

Figure S1. The *dnaE915* DinB-mediated survival loss is strain independent. (**A**) *dnaE915*Δ*dinB*/p*dinB*$^{+}$ MG1655 cells are hypersensitive to MMS (7.5 mM). The percent survival is calculated based on the CFU/mL of the untreated cells, which is similar for all strains (~1x10^{9} CFU/mL). Error bars represent the standard deviation of the mean from the analysis of at least 3 independent isolates. (**B**) Synthetic sick phenotypes are also observed in the MG1655 strain.Comparison of representative Δ*dinBdnaE915* colonies with pVector or p*dinB(D103N)* (magnification 40X) or DAPI (live) and propidium iodide (dead) staining of cells from the respective colonies (1000X).

Figure S2. The *dnaE915* DinB-mediated survival loss is independent of *sulA*. (**A**) Δ*sulAdnaE915*Δ*dinB*/p*dinB*$^{+}$ cells are hypersensitive to MMS (7.5 mM). The percent survival is calculated based on the CFU/mL of the untreated cells, which is similar for all strains (~1x10^{9} CFU/mL). Error bars represent the standard deviation of the mean from the analysis of at least 3 independent isolates. (**B**) Cultures started from Δ*sulAdnaE915ΔdinB/*p*dinB(D103N)* colonies do not reach saturation after 24 hrs in LB rich medium. There is a large variation in saturation of these cultures compared to the isogenic *sulA*$^{+}$ strain. Perhaps suppressors are acquired quicker in this strain. (**C**) Representative colony sections and microscopy of cells from the Δ*sulAdnaE915*Δ*dinB* strain with the vector or p*dinB(D103N)*. In Δ*sulAdnaE915*Δ*dinB/pdinb(D103N)* colonies have an altered morphology and the cells are elongated, with fewer survivors and increased SOS induction when compared to cells carrying the vector. All strains carry a green fluorescent protein as a reporter of SOS induction (Pr-*sulA*-GFP). The quantification shown below each micrograph is from the count of at least 500 cells from different fields of at least 3 independent colonies at 1000X.

Figure S3. DinB(D103N) dependent synthetic sickness, but not DinB-dependent survival loss upon MMS treatment, occurs in absence of SOS induction. (**A**) DinB dependent MMS hypersensitivity is abolished in SOS deficient cells. The percent survival is calculated based on the CFU/mL of the untreated cells, which is similar for all strains (~1x10^{9} CFU/mL). Error bars represent the standard deviation of the mean from the analysis of at least 3 independent isolates. (**B**) Colonies formed by *dnaE915*Δ*dinB*/p*dinB(D103N)* that are unable to induce the SOS network due to Δ*recA* or *lexA3* alleles have altered morphology and contain fewer cells. Representative sections of colonies of SOS deficient *dnaE915*Δ*dinB* with pVector (normal morphology) or p*dinB(D103N)* (synthetically sick) at 40X. (**C**) Saturated cultures of SOS deficient *dnaE915*Δ*dinB* p*dinB(D103N)* colonies grown in LB rich medium have ~10 fold less CFUs than isogenic strains carrying the vector. (**D**) SOS induction of cells within colonies was determined using FACS. *ΔrecA* and *lexA3* alleles abolish SOS induction in *dnaE915*Δ*dinB/*p*dinB(D103N)* cells. All strains carry a Pr-*sulA*-GFP construct to detect SOS induction. Cells with fluorescence (FITC-A) >10^{2}

are considered to have SOS induction. The data shown is from the analyses of 10,000 cells from 3 independent colonies of each strain.

Figure S4. The β-processivity clamp binding motif on DinB is required to observe survival loss in the *dnaE915* strain. (**A**) Cultures started from *dnaE915*Δ*dinB/*p*dinB(D103N)*Δβ colonies reach saturation after 24 hrs in LB broth just as cells carrying the vector. Error bars represent the standard deviation of the mean from the analysis of at least 5 independent isolates. (**B**) Representative colony sections and fields of view of cells from *dnaE915*Δ*dinB* strain with p*dinB(D103N)*Δβ. *dnaE915*Δ*dinB/*p*dinB(D103N)*Δβ has similar colony morphology, cell size, and fraction of dead cells as cells carrying the vector. The quantification is from the count of at least 500 cells from different fields and independent isolates at 1000X.

Figure S5. Genetic interactions between *dnaE915* and the catalytically inactive p*dinB(D103N)* inhibit colony formation on minimal medium. Δ*dinBdnaE915*/p*dinB(D103N)* is unable to form colonies on M9 glucose minimal medium, with or without casamino acid supplementation. This phenotype is independent of *sulA*. Deletion of *recA* or the β-clamp binding motif of *dinB(D103N)* allows for the formation of colonies on minimal medium. Representative plates are shown.

Figure S6. Strong selective pressure against antagonistic genetic interactions between *dnaE915* and plasmid borne *dinB* alleles allows for suppressor isolation. (**A**) The inability of the synthetic sick *dnaE915*Δ*dinB/*p*dinB(D103N)* strain to reach saturation in LB medium after 24 hrs subsides upon subculturing. (**B**) *dnaE915*Δ*dinB* p*dinB*$^{+}$ cultures started from colonies coming from MMS-hypersensitive cultures (7.5mM) are no longer MMS hypersensitive upon re-treatment with MMS (7.5 mM; gray bars) or NFZ (7.5 μM; white bars). "p*dinB* truncation" refers to a plasmid with an out of frame *dinB* gene deletion, between basepairs 353-366, which encodes a 151 amino acid truncated protein. The percent survival is calculated based on the CFU/mL of the untreated cells, which is similar for all strains (~1x10^{9} CFU/mL). The mutations were identified as intragenic or extragenic after DNA sequencing. Error bars represent the standard deviation of the mean from the analysis of at least 3 independent isolates.

Figure S7. Mutations suppress DinB-mediated growth defects in Δ*dinBdnaE915*. (**A**) Cultures of Δ*dinBdnaE915* with plasmid borne wild-type *dinB*$^{+}$, *dinB(F292Y)*, *dinB(V7G)*, or *dinB(V288G)* were treated with DNA damaging agents (NFZ (7.5 μM: white bars) or MMS (7.5 mM: gray bars)) to identify suppression of MMS dependent survival loss. The percent survival is calculated based on the CFU/mL of the untreated cells, which is similar for all strains (~1x10^{9} CFU/mL). Error bars represent the standard deviation of the mean from the analysis of at least 3 independent isolates. (**B**) F292Y, V7G, or V288G amino acid substitutions suppress *dinB(D103N)* synthetic sickness in

untreated Δ*dinBdnaE915* cells. Representative Δ*dinBdnaE915* colonies carrying the indicated plasmids at 40X magnification.

Figure S8. The DinB(F292Y) variant maintains a structure similar to that of wild-type DinB. (**A**) Circular dichroism (CD) reveals that the secondary structure of DinB(F292Y) is similar to that of wild-type DinB. (**B**) The secondary structure composition of DinB(F292Y) is similar to DinB. αR=Fraction of regular alpha helices, αD=Fraction of disordered alpha helices, βR=Fraction of regular beta sheets, βD=Fraction of disordered beta sheets, T=Fraction of turns, U= Fraction of undefined or unstructured elements.

Figure S1

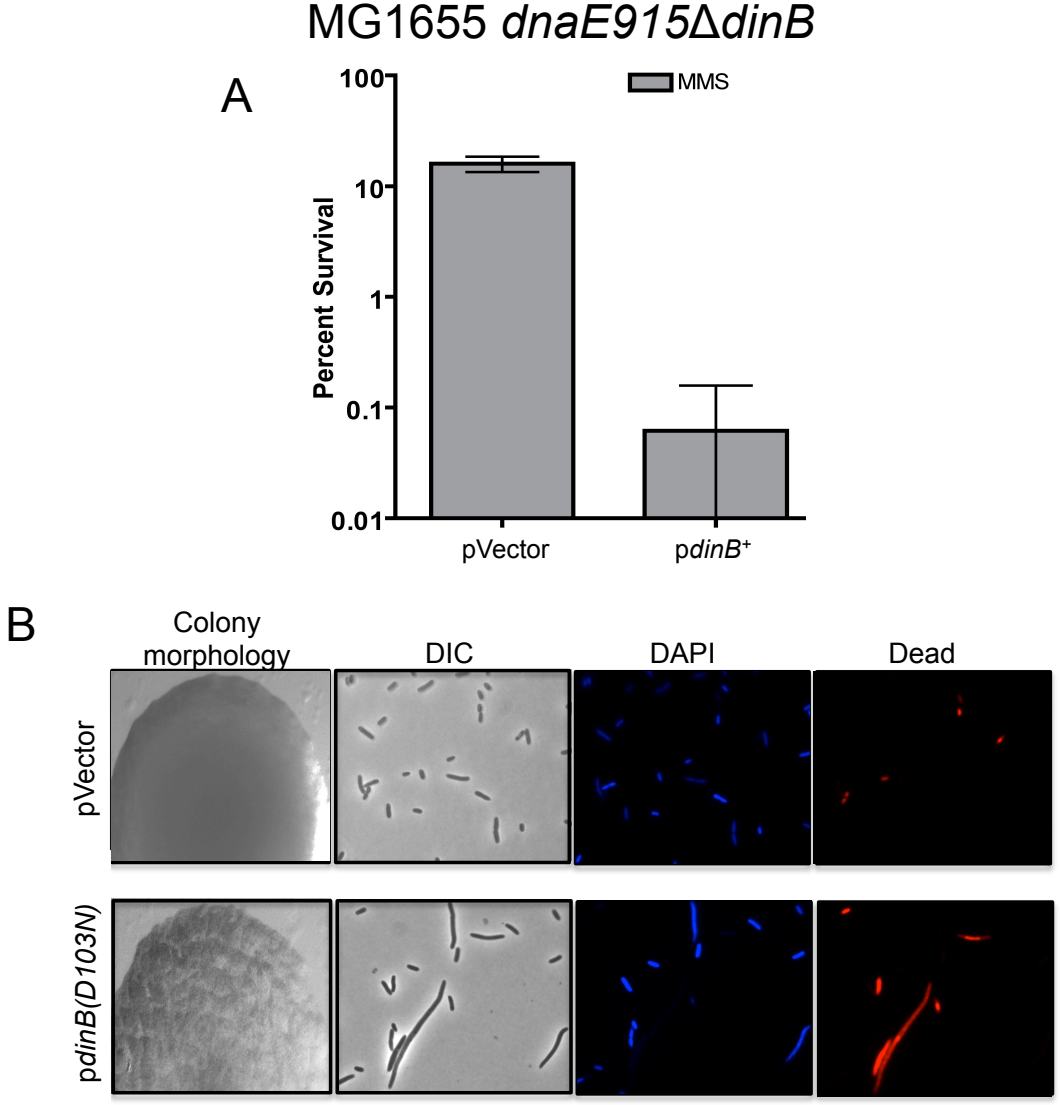

MG1655 dnaE915ΔdinB
A
MMS
100
10
1
0.1
0.01
Percent Survival
pVector
pdinB+
B
Colony morphology
DIC
DAPI
Dead
pVector
pdinB(D103N)

Figure S2

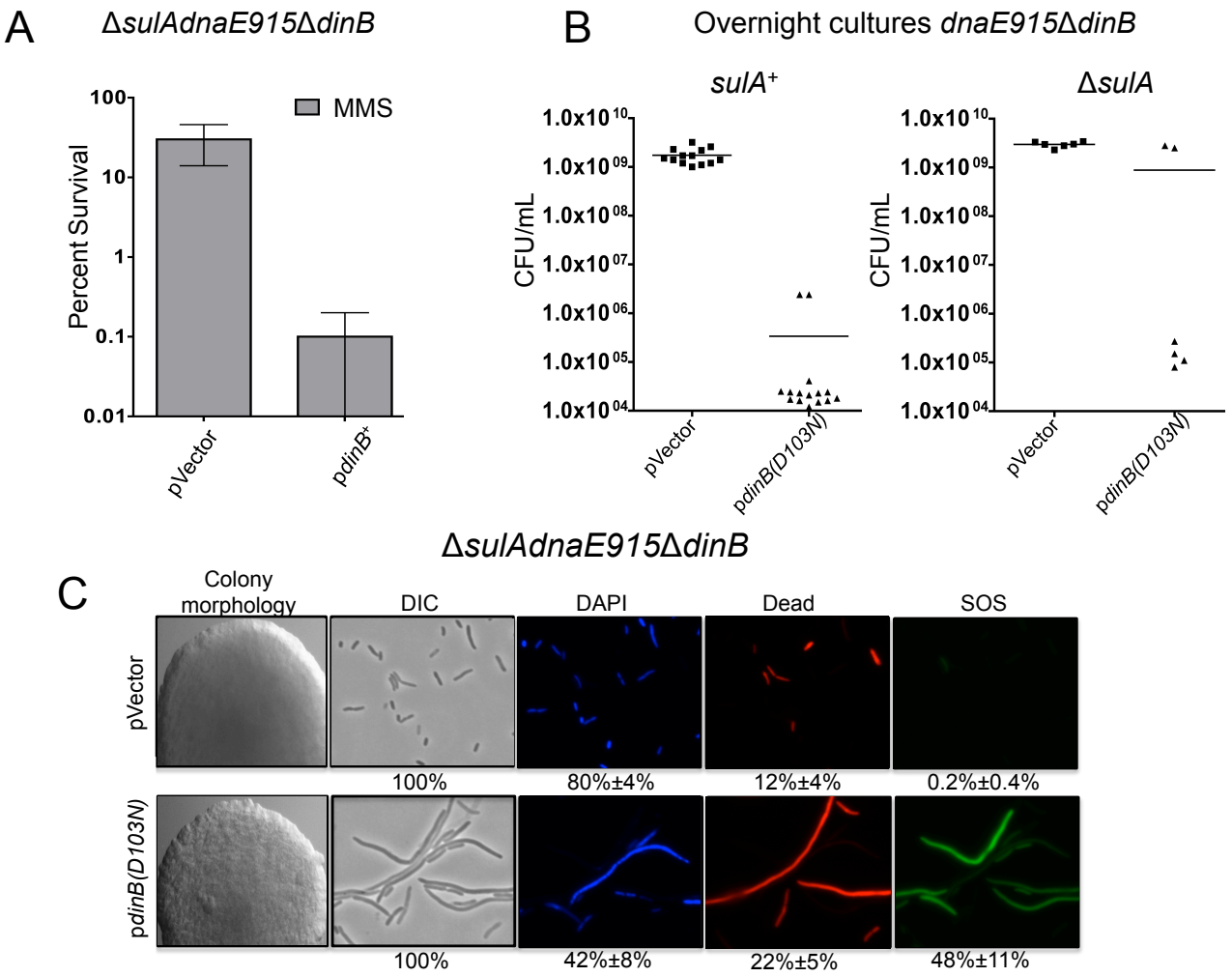

A
ΔsulAdnaE915ΔdinB
MMS
Percent Survival
100
10
1
0.1
0.01
pVector
pdinB+
B
Overnight cultures dnaE915ΔdinB
sulA+
ΔsulA
CFU/mL
1.0x10 10
1.0x10 09
1.0x10 08
1.0x10 07
1.0x10 06
1.0x10 05
1.0x10 04
pVector
pdinB(D103N)
ΔsulAdnaE915ΔdinB
C
Colony morphology
DIC
DAPI
Dead
SOS
pVector
pdinB(D103N)
100%
80%±4%
12%±4%
0.2%±0.4%
100%
42%±8%
22%±5%
48%±11%

Figure S3

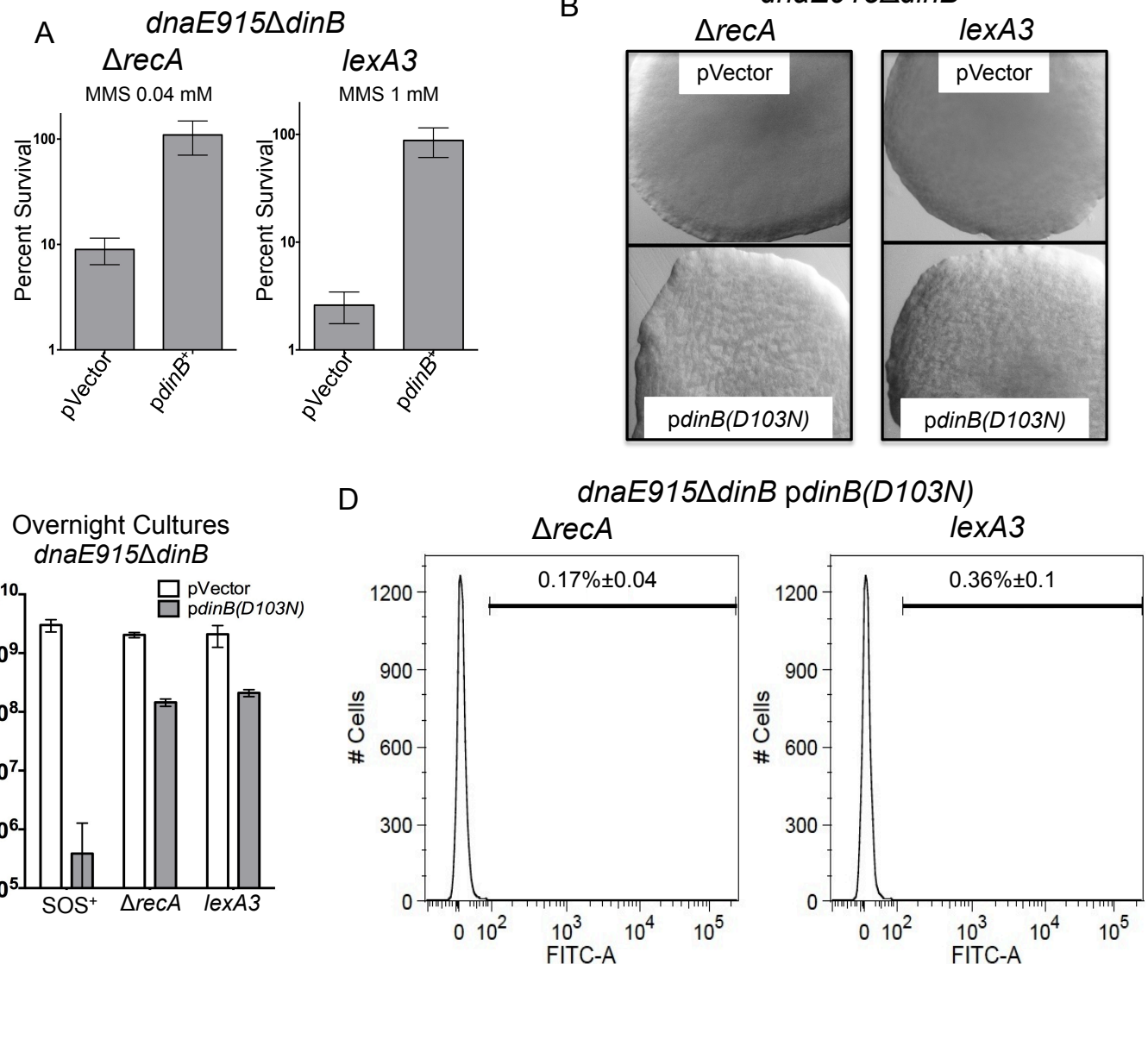

A
dnaE915ΔdinB
ΔrecA
lexA3
MMS 0.04 mM
MMS 1 mM
Percent Survival
100
10
1
pVector
pdinB+
B
dnaE915ΔdinB
ΔrecA
lexA3
pVector
pdinB(D103N)
C
Overnight Cultures
dnaE915ΔdinB
pVector
pdinB(D103N)
CFU/mL
1×10^10
1×10^9
1×10^8
1×10^7
1×10^6
1×10^5
SOS+
ΔrecA
lexA3
D
dnaE915ΔdinB pdinB(D103N)
ΔrecA
lexA3
0.17%±0.04
0.36%±0.1
Cells
1200
900
600
300
0
0 10^2 10^3 10^4 10^5
FITC-A

Figure S4

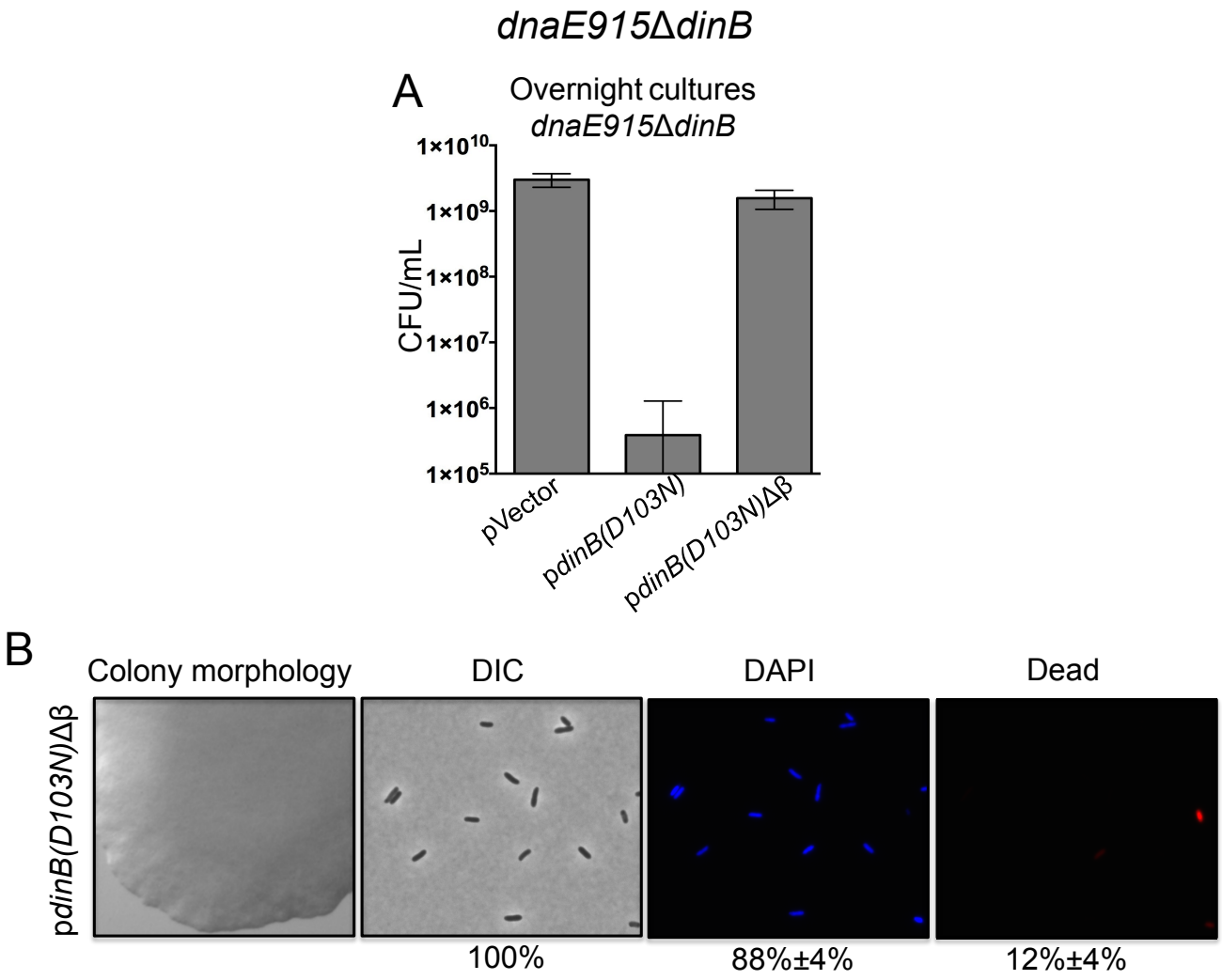

dnaE915ΔdinB
A
Overnight cultures
dnaE915ΔdinB
CFU/mL
1×10^10
1×10^9
1×10^8
1×10^7
1×10^6
1×10^5
pVector
pdinB(D103N)
pdinB(D103N)Δβ
B
Colony morphology
DIC
DAPI
Dead
pdinB(D103N)Δβ
100%
88%±4%
12%±4%

Figure S5

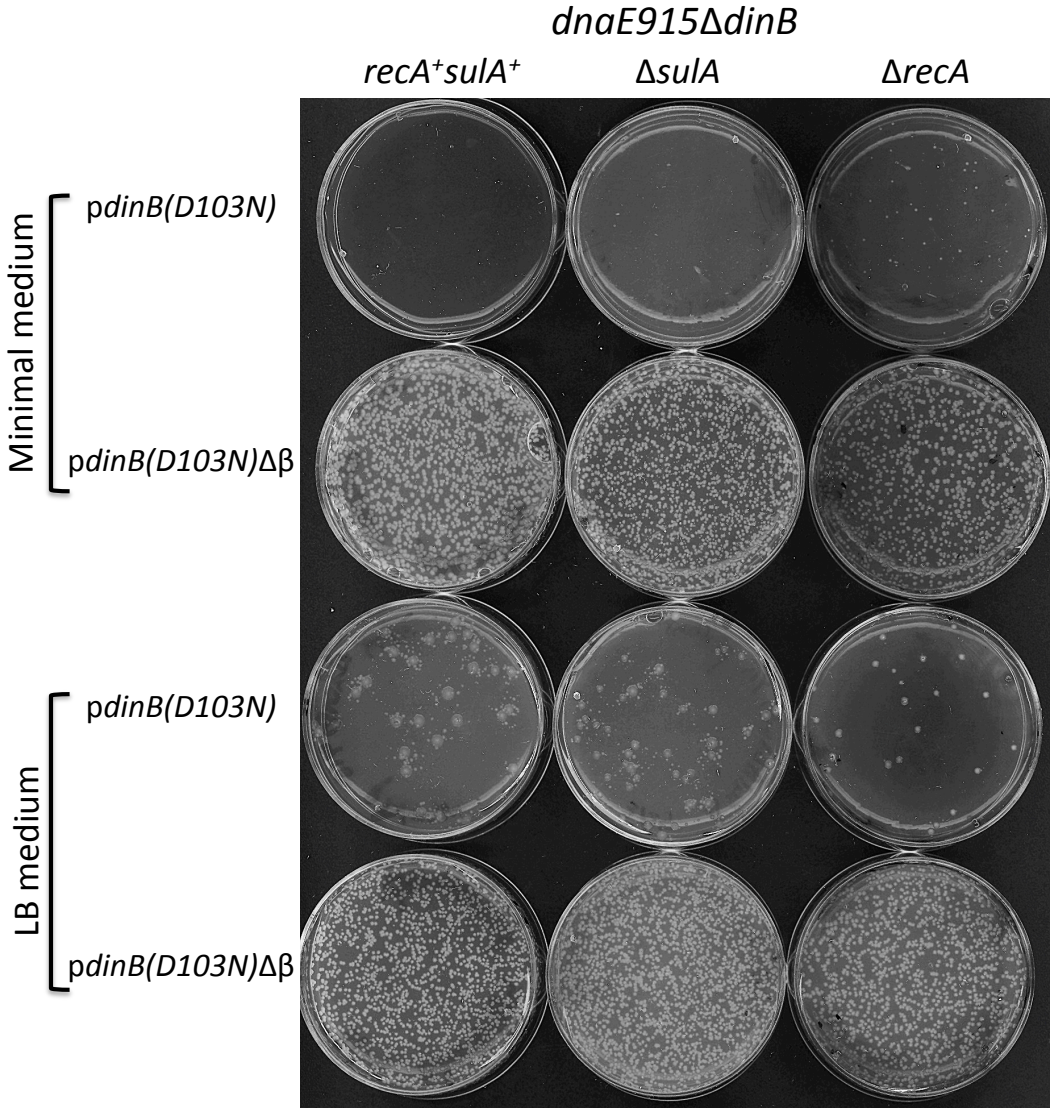

dnaE915ΔdinB
recA+sulA+
ΔsulA
ΔrecA
Minimal medium
pdinB(D103N)
pdinB(D103N)Δβ
LB medium
pdinB(D103N)
pdinB(D103N)Δβ

Figure S6

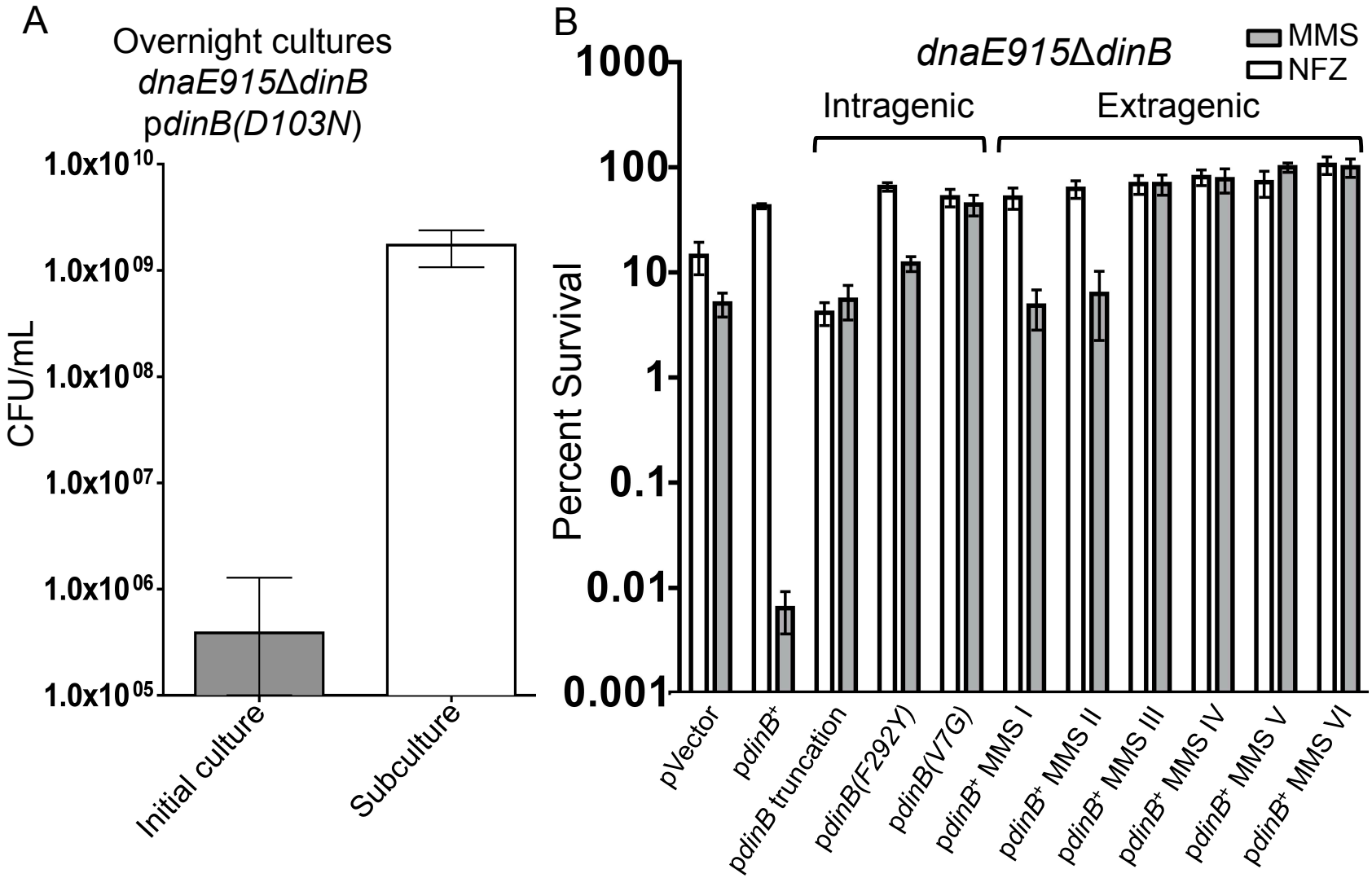
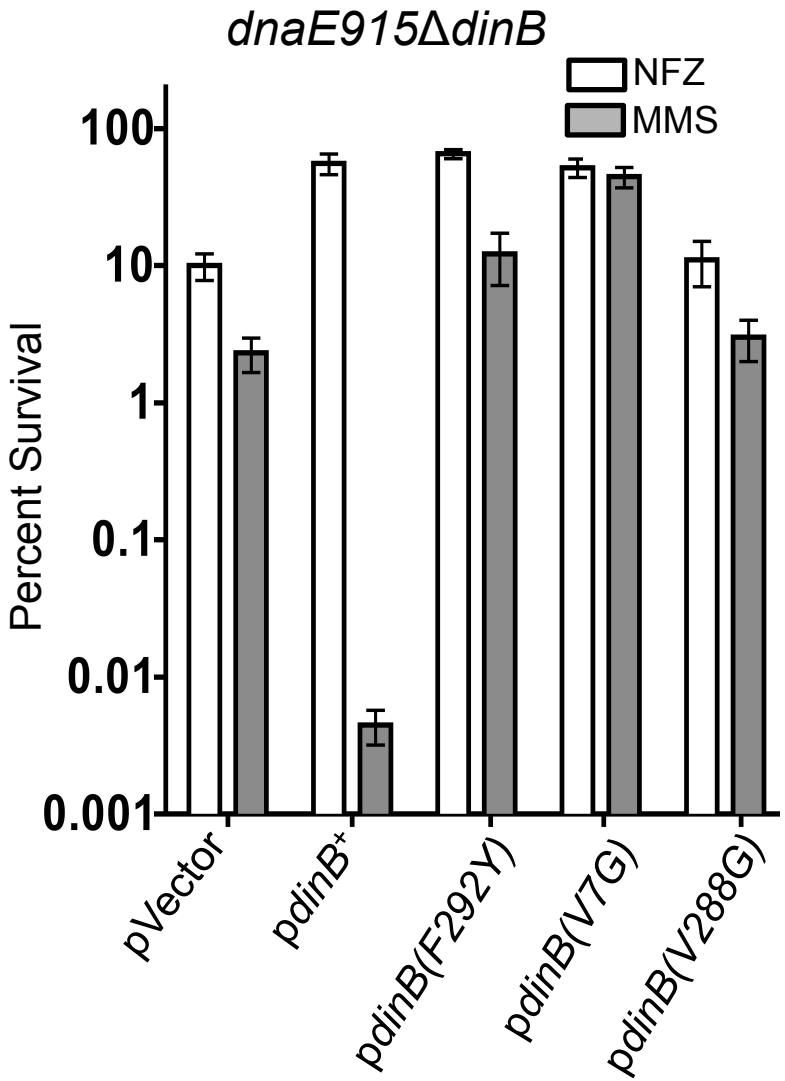

A
Overnight cultures
dnaE915ΔdinB
pdinB(D103N)
CFU/mL
1.0x10 10
1.0x10 09
1.0x10 08
1.0x10 07
1.0x10 06
1.0x10 05
Initial culture
Subculture
B
dnaE915ΔdinB
MMS
NFZ
Intragenic
Extragenic
Percent Survival
1000
100
10
1
0.1
0.01
0.001
pVector
pdinB+
pdinB truncation
pdinB(F292Y)
pdinB(V7G)
pdinB+ MMS I
pdinB+ MMS II
pdinB+ MMS III
pdinB+ MMS IV
pdinB+ MMS V
pdinB+ MMS VI

Figure S7

A


dnaE915ΔdinB
NFZ
MMS
Percent Survival
100
10
1
0.1
0.01
0.001
pVector
pdinB+
pdinB(F292Y)
pdinB(V7G)
pdinB(V288G)

B

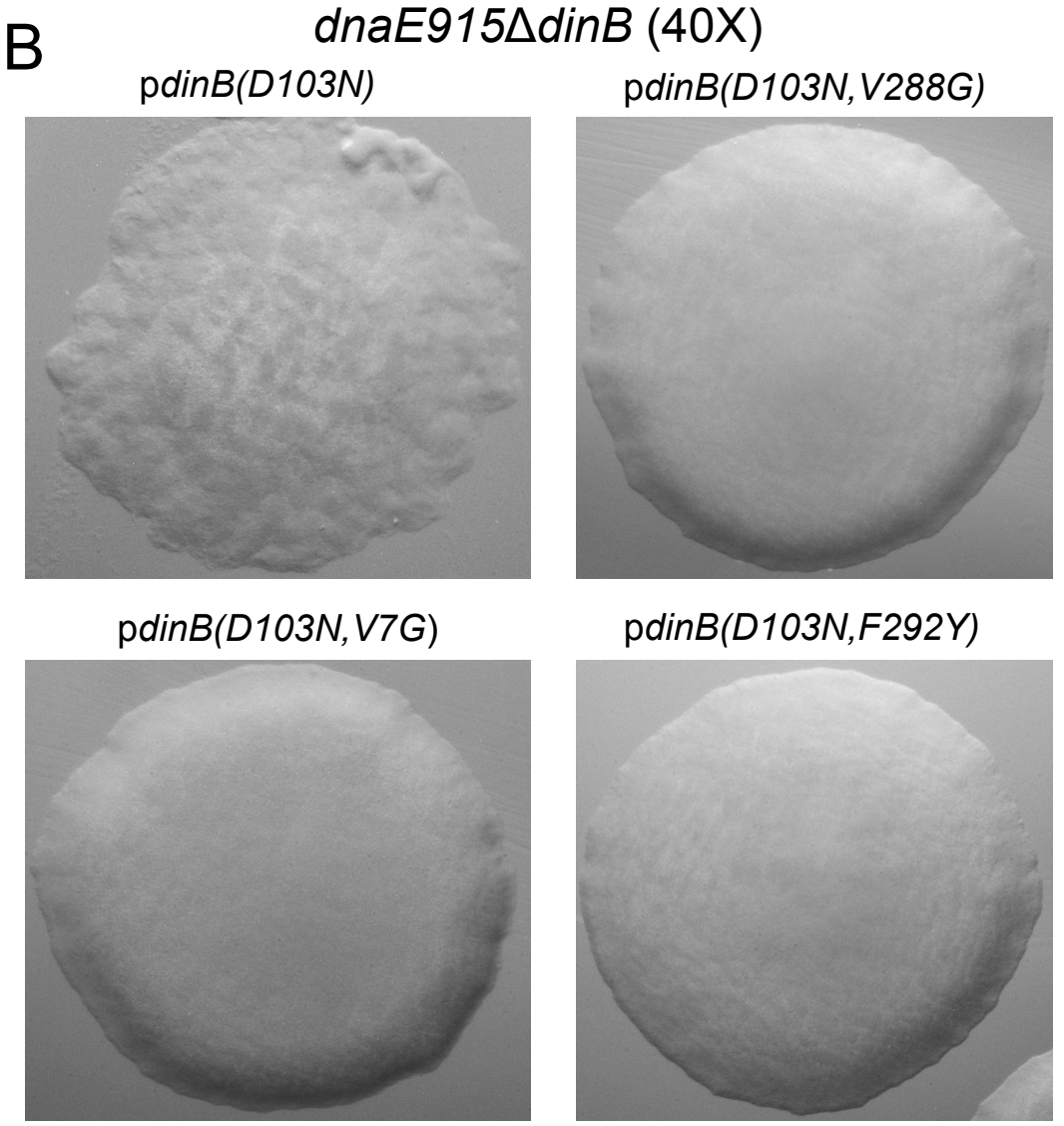
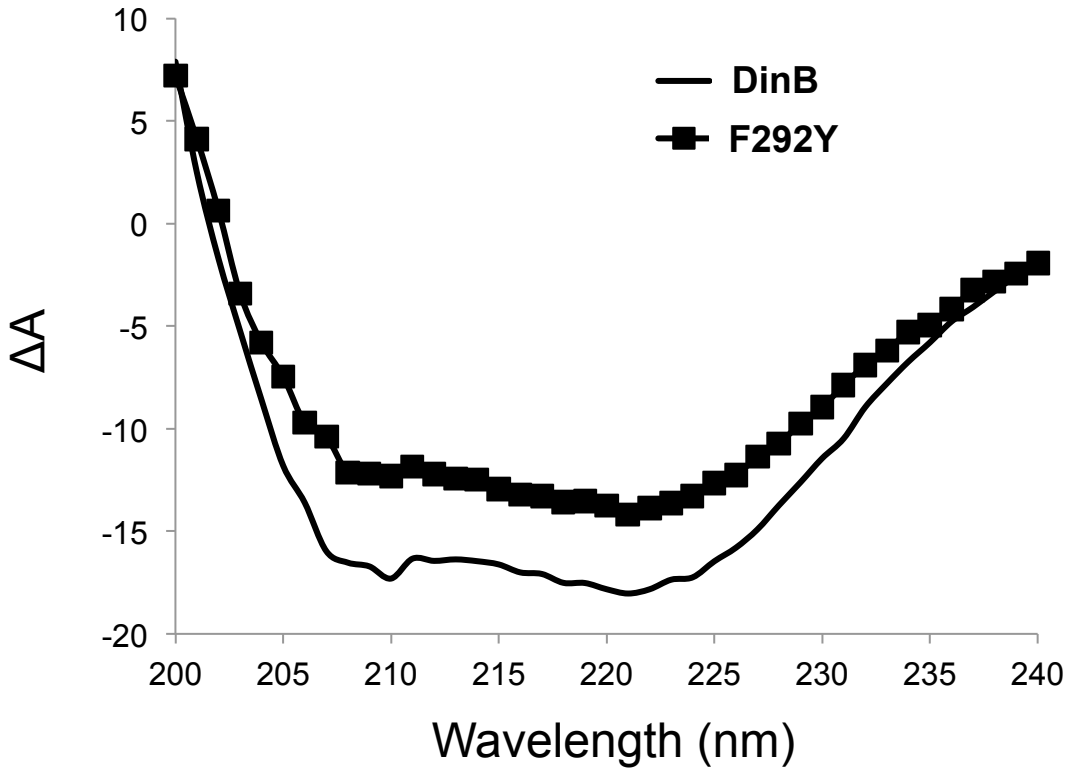

dnaE915ΔdinB (40X)
pdinB(D103N)
pdinB(D103N,V288G)
pdinB(D103N,V7G)
pdinB(D103N,F292Y)

Figure S8

A



B

	αR	αD	βR	βD	T	U
DinB	0.796	0.192	0	0	0	0.012
DinB(F292Y)	0.727	0.248	0	0	0.001	0.024