SUPPORTING INFORMATION

Cryptic protein interactions regulate DNA replication initiation

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Running title: Cryptic interactions regulate replication initiation

Supplementary Methods

Building the *dnaD23* strain using CRISPR/Cas gene-editing. Note that the sequences of all primers outlined in this section can be found in Table S1. The proto-spacer was created by annealing 50 µL of 20 µM oLM462 with 50 µL of 20 µM oLM463 and boiling for 10 minutes followed by slow cooling. The 5' ends of the annealed oligos were phosphorylated using T4 Polynucleotide Kinase (PNK) at 37°C for 30 minutes. PNK was heat inactivated at 65°C for 20 minutes. The pPB41 vector was digested with BsaI at 37°C for 20 minutes, followed by heat inactivation at 65°C for 20 minutes. The 5' phosphate groups from the vector were removed by treating with Calf Intestinal Phosphatase (CIP) at 37°C for 1 hour and gel purifying the digested vector. The proto-spacer was ligated into the prepared pPB41 vector using T4 DNA Ligase at room temperature for 2 hours. $CaCl_2$-competent MC1061 cells were transformed with 10 µL of the reaction and transformants were selected by plating on LB agar with 100 µg mL^{-1} ampicillin.

The editing template (containing the A166T and silent mutations destroying the PAM site) was created using the primer extension method. Fragments of *dnaD* were amplified from PY79 genomic DNA in three pieces to introduce the mutations as follows: PCR1 (oLM468 and oLM465); PCR2 (oLM464 and oLM467); and PCR3 (oLM466 and oLM469). The amplified products were gel purified and mixed followed by amplifying with the flanking primers (oLM468 and oLM469) to make the completed template. The pPB41 vector containing the proto-spacer was amplified in two parts using primers oPEB217 and oPEB218 as well as oPEB232 and oPEB234 (see Table S1 for sequences). The amplified vector components were then assembled with the editing template using Gibson

assembly to make pLM190. Plasmids were isolated from single colonies and verified using the University of Michigan Sequencing Core (https://seqcore.brcf.med.umich.edu/).

To modify the *B. subtilis* genome, 1 µg of pLM190 was incubated with 200 µL of competent PY79 cells for 1 hour at 37°C. The cells were plated onto LB agar with 100 µg mL^{-1} spectinomycin and incubated overnight at 30°C. Colonies were restreaked on LB agar with 100 µg mL^{-1} spectinomycin and incubated for a second overnight at 30°C. Colonies were then restreaked on LB agar without antibiotics and incubated overnight at 37°C. As a final test, colonies were restreaked on both LB agar and LB agar with 100 µg mL^{-1} spectinomycin overnight at 30°C and 48°C to find cells that are sensitive to spectinomycin and high temperatures. The final strain was incubated in 1 mL of LB media until an OD_{600} of 1.0 and then mixed with 0.25 mL of 50% glycerol prior to flash freezing in liquid nitrogen and storage at -80°C.

B2H using selective growth on minimal medium containing maltose. Minimal media plates were prepared fresh on the day of the experiment. The plates consisted of 1X M63 media supplemented with 50 µg mL^{-1} ampicillin, 25 µg mL^{-1} kanamycin, 0.5 mM IPTG, 1 mM $MgSO_4$, ($5x10^{-5}$)% thiamine, 0.2% D-maltose, and 0.04% casamino acids. Co-transformants were streaked onto the minimal media plates in an “X” pattern and the plates were then incubated for 3 days at 30°C.

Table S1. Primers Used to Construct Plasmids

Primer	Sequence (5'-3')
oLM1	TCGAGCACCACCACCACCAC
oLM2	ACCTCCAATCTGTTCGCGGTG
oLM5	ACCGCGAACAGATTGGAGGTCTTTATACCATTTTTGAGGAAGAGTTCGCAAG
oLM6	GTGGTGGTGGTGGTGCTCGATTATTGTTCAAGCCAATTGTAAAAAGGAACCTGC
oLM29	ACAGAGAACAGATTGGTGGAATGAAAAAACAGCAATTTATTGATATGC AGGAG
oLM30	AGCTCGAATTCGGATTATTAGCTTTTTTGTTCCCCTTCTGCTTTTC
oLM125	GTGGTGGTGGTGGTGCTCGATTATTGTTCGCCTTCACGCAGATG
oLM126	ACCGCGAACAGATTGGAGGTGAAGAGGATTCTCTGGATGGGAAGC
oLM127	GTGGTGGTGGTGGTGCTCGATTAATAGGCAGAGTATTTTTTCAGTTT TTGCATTTC
oLM129	ACCGCGAACAGATTGGAGGTCTTGCCGAGGGGTCCG
oLM140	TAACTAAGTAATATGGTGCACTCTCAGTACAATCTG
oLM141	GCTCGGTACCCGGGGATC
oLM146	TAACTAAGAATTCGGCCGTCGTTTTACAAC
oLM147	CTTAGGTACCCGGGGATCCTC
oLM176	GACGGCCGAATTCTTAGTTAGCTTTTTTGTTCCCCTTCTGCTTTTC
oLM180	AGGATCCCCGGGTACCGAGCATGAAAAAACAGCAATTTATTGA TATGCAGGAG
oLM181	TGCACCATATTACTTAGTTATTGTTCAAGCCAATTGTAAAAAGG AACCTG
oLM182	TGCACCATATTACTTAGTTAGCTTTTTTGTTCCCCTTCTGCTTTTC
oLM183	AGGATCCCCGGGTACCGAGCCTTTATACCATTTTTGAGGAAGAG TTCGCAAG
oLM197	ATGTATACCTCCTTAGGATCCCATTTCCCCCTTTG
oLM198	GATCCGAGCTCCCGCGGGCG

Table S1 Continued

Primer	Sequence (5'-3')
oLM203	AGGATCCCCGGGTACCTAAGATGGCTGACTATTGGAAAGATGTACTGC
oLM204	GACGGCCGAATTCTTAGTTAATAGGCAGAGTATTTTTTCAGTTTTTG CATTTCTTC
oLM211	CATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCC
oLM212	AGCTTGCATGCCTGCAGGTCGACTC
oLM215	TCACACAGGAAACAGCTATGCTTTATACCATTTTTGAGGAAGAGTTCGCA AGACCG
oLM216	GACCTGCAGGCATGCAAGCTTTGTTCAAGCCAATTGTAAAAAGGAACCTG CCTTTTATAC
oLM217	CATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTC
oLM218	AGCTTGCATGCCTGCAGGTCGACTC
oLM308	AGGATCCCCGGGTACCGAGCAATCTTCTGCTCACGCATTATAAA CAGCTTGGGC
oLM309	GATCCTAAGGAGGTATACATATGAAAAAACAGCAATTTATTGATATGCA GGAGCAGGG
oLM310	CCGCGGGAGCTCGGATCTTATTATTGTTCAAGCCAATTGTAAAAAGGA ACCTGCC
oLM327	TCACACAGGAAACAGCTATGGCTGACTATTGGAAAGATGTACTGCC TGTAGACC
oLM328	GACCTGCAGGCATGCAAGCTATCTTCTGAAATGGCCGGGTGCG
oLM348	AGGATCCCCGGGTACCTAAGATGGAAAATATATTAGACCTGTG GAACCAAGCCC
oLM349	GACGGCCGAATTCTTAGTTACTATTTAAGCTGTTCTTTAATTTCT TTTACATGCTGCTG
oLM355	GACCTGCAGGCATGCAAGCTATAGGCAGAGTATTTTTTCAGTTTTTG CATTTCTTCC
oLM360	GGATCATATGCTCCTACACCGAATCAGCTG
oLM361	CAGCTGATTCGGTGTAGGAGCATATGATCC
oLM365	GACGGCCGAATTCTTAGTTATTGAGGAATGACAAACTTAATGCT CAATTCTTCCCC
oLM368	AGGATCCCCGGGTACCTAAGATGCTCAATCCAAAATATACTTTTG ATACTTTTGTCATC
oLM369	GACGGCCGAATTCTTAGTTATGAATAAGCGACAACTCTGATTAAT GCTCCTTCG
oLM371	AGGATCCCCGGGTACCTAAGGCTGATCTGGCCGCTGAGGC

Table S1 Continued

Primer	Sequence (5'-3')
oLM372	TCACACAGGAAACAGCTATGCTTGCCGAGGGGTCCGAGTATACTTC
oLM373	GACCTGCAGGCATGCAAGCTTTGTTCGCCTTCACGCAGATGCAC
oLM376	TCACACAGGAAACAGCTATGGAAGAGGATTCTCTGGATGGGAAGCTG ATTGC
oLM397	GCGAACAGATTGGAGGTATGGAAAATATATTAGACCTGTGGAACCAAG CCCTTGCTC
oLM398	GTGGTGGTGGTGCTCGATTATTGAGGAATGACAAACTTAATGCTCAAT TCTTCCCCG
oLM421	ACAGAGAACAGATTGGTGGAAATCTTCTGCTCACGCATTATAAACAGC TTGGGC
oLM422	ACAGAGAACAGATTGGTGGAATGGCTGACTATTGGAAAGATGTACTGCCTG
oLM423	AGCTCGAATTCGGATTATTAATCTTCTGAAATGGCCGGGTGCG
oLM462	AAACTCAGGAAAACTCAGTTTCCGCTACATTGACG
oLM463	AAAACGTCAATGTAGCGGAAACTGAGTTTTCCTGA
oLM464	GCACAACTGATCAAACACACGTTAAAAGAGGCTG
oLM465	CAGCCTCTTTTAACGTGTGTTTGATCAGTTGTGC
oLM466	CTCAGTTTCCGCTACATTGATAGAATTTTGTTTGAATGGAAG
oLM467	CTTCCATTCAAACAAAATTCTATCAATGTAGCGGAAACTGAG
oLM468	CTGAATTCGTAATGAGGTTCATGAAAAAACAGCAATTTATTGATATGCA GGAGCAGGG
oLM469	ACCAAGCCTATGCCTACAGCACTTCCAGAACCGAGTCCTTCCACCG
oLM482	TTATTGAAGAATGCGGCTCATTTGAGAAATATTCTCTTCAGCCTT TATGGGGCAAGC
oLM483	GAATATTTCTCAAATGAGCCGCATTCTTCAATAAACAGAAAGCCTT TTTGAATAAACATC
oLM492	GCAAACCGAGTTTTGAGGCTTGGATGAAGTC
oLM493	GACTTCATCCAAGCCTCAAAACTCGGTTTGC
oLM494	CCGAGTTTTGAGACTGCGATGAAGTCAACC
oLM495	GGTTGACTTCATCGCAGTCTCAAAACTCGG

Table S1 Continued

Primer	Sequence (5'-3')
oLM496	CCCAATGAAGCTGCCAGAGACTGGC
oLM497	GCCAGTCTCTGGCAGCTTCATTGGG
oLM509	CAAACCGAGTTTTGAGTCTTGGATGAAGTCAACC
oLM510	GGTTGACTTCATCCAAGACTCAAAACTCGGTTTG
oLM511	CGGCTCCCGCTGAATTTGCCAG
oLM512	CTGGCAAATTCAGCGGGAGCCG
oLM519	CAAAAAGGCTTTCTGTTTGCTGAAGAATGCGAGGATC
oLM520	GATCCTCGCATTCTTCAGCAAACAGAAAGCCTTTTTG
oLM521	CAGATTGCGGATGTTTGCTCAAAAAGGCTTTCTGTTTATTG
oLM522	CAATAAACAGAAAGCCTTTTTGAGCAAACATCCGCAATCTG
oLM525	GATCCTAAGGAGGTATACATATGAATCTTCTGCTCACGCATTATAAACA GCTTGGG
oLM546	GGCTCCCAATGAATATGCCAGAGACTGGC
oLM547	GCCAGTCTCTGGCATATTCATTGGGAGCC
oLM548	GGCTCCCAATGAATTTGTCAGAGACTGGC
oLM549	GCCAGTCTCTGACAAATTCATTGGGAGCC
oLM578	TCACACAGGAAACAGCTATGGAACCAATCGGCCGTTCCCTGC
oLM579	GACCTGCAGGCATGCAAGCTCTGGATATACATGCTTTTCATAAGAGAC TGTTGTTTCTTC
oLM582	TCACACAGGAAACAGCTATGCAGGATCTTCTTGGAGCGACGTTCC
oLM583	GACCTGCAGGCATGCAAGCTTGGATGTCGGCGGTTTTCTCCG
oLM590	AGGATCCCCGGGTACCTAAGATGTCGGAAAAAGAAATTTGGGAAAAAG TGCTTG
oLM591	GACGGCCGAATTCTTAGTTATAATTCTTCAGTAGTAATAAAGTGAGGTTT AACTTCATAG
oLM592	GCTGTAAGTTACTCAGCGTTCCTAAAAGATACTGAGCTTTACAC

Table S1 Continued

Primer	Sequence (5'-3')
oLM593	GTGTAAAGCTCAGTATCTTTTAGGAACGCTGAGTAACTTACAGC
oLM594	CTGTAAGTTACTCAACTGCGCTAAAAGATACTGAGCTTTAC
oLM595	GTAAAGCTCAGTATCTTTTAGCGCAGTTGAGTAACTTACAG
oLM596	GTATTATCGAGTATTCCTGCGAATGCAAATTGGTTAAATCAACAATATG
oLM597	CATATTGTTGATTTAACCAATTTGCATTCGCAGGAATACTCGATAATAC
oLM600	AGGATCCCCGGGTACCGAGCATGGATAAATATCAATTAAAAGCAAGA CCTGTAGTGATAC
oLM601	TGCACCATATTACTTAGTTATTGTTTAAATTTTTCTTTACTATTTAGCTC TTTCGTTTCC
oLM602	AGGATCCCCGGGTACCTAAGATGTCACTTTCGCTTTGGCAGCAGTG
oLM603	GACGGCCGAATTCTTAGTTATGCCGCTTGTGGCGTTTGC
oLM606	GCAAACCGAGTTTTGAGATGTGGATGAAGTCAACCAAAGCCCACTC
oLM607	TTGGTTGACTTCATCCACATCTCAAAACTCGGTTTGCTCAACTTTTTTTCG
oLM634	AGGATCCCCGGGTACCGAGCATGGCTGACTATTGGAAAGATGTACTGC CTGTAG
oLM635	TGCACCATATTACTTAGTTAATCTTCTGAAATGGCCGGGTGCGTAAAAA ATTG
oLM636	AGGATCCCCGGGTACCGAGCCTTGCCGAGGGGTCCGAGTATACTTCTG
oLM637	TGCACCATATTACTTAGTTATTGTTCGCCTTCACGCAGATGCACCG
oLM638	AGGATCCCCGGGTACCGAGCGAAGAGGATTCTCTGGATGGGAAGCTGA TTGC
oLM639	TGCACCATATTACTTAGTTAATAGGCAGAGTATTTTTTCAGTTTTTGCAT TTCTTCC
oLM640	AGGATCCCCGGGTACCGAGCATGGAACCAATCGGCCGTTCCCTG
oLM641	TGCACCATATTACTTAGTTACTGGATATACATGCTTTTCATAAGAGACTG TTGTTTC
oLM642	AGGATCCCCGGGTACCGAGCCAGGATCTTCTTGGAGCGACGTTCCAG
oLM643	TGCACCATATTACTTAGTTATGGATGTCGGCGGTTTTCTCCGTC
oLM644	ATTTCACACAGGAAACAGCTATGAAAAAACAGCAATTTATTGATATGCA GGAGCAGGG

Table S1 Continued

Primer	Sequence (5'-3')
oLM645	GACCTGCAGGCATGCAAGCTGCTTTTTTGTTCCCCTTCTGCTTTTCTTTCCTG
oPEB217	GAACCTCATTACGAATTCAGCATGC
oPEB218	GAATGGCGATTTTCGTTCGTGAATAC
oPEB232	GCTGTAGGCATAGGCTTGGTTATG
oPEB234	GTATTCACGAACGAAAATCGCCATTCCTAGCAGCACGCCATAGTGACTG
oJS638	TAATAATCCGAATTCGAGCTCCGTCGAC
oJS639	TCCACCAATCTGTTCTCTGTGAGCC

Table S2. Plasmid List for B2H Assay

Plasmid	Vector	Description[†]	Primers[‡]
BsDnaA Plasmids			
pLM137	pKT25	$DnaA^{FL}$ (1-446)	oLM348 + oLM349
pLM146	pKT25	$DnaA^{DI}$ (1-82)	oLM348 + oLM365
pLM148	pKT25	$DnaA^{DIII}$ (111-326)	oLM368 + oLM369
pLM150	pKT25	$DnaA^{DIV}$ (335-446)	oLM371 + oLM349
pLM211	pKT25	$DnaA^{DI}$ T26A (1-82)	(**oLM348** + oLM493) + (oLM492 + **oLM365**)[§]
pLM212	pKT25	$DnaA^{DI}$ W27A (1-82)	(**oLM348** + oLM495) + (oLM494 + **oLM365**)[c]
pLM213	pKT25	$DnaA^{DI}$ F49A (1-82)	(**oLM348** + oLM497) + (oLM496 + **oLM365**)[§]
pLM220	pKT25	$DnaA^{DI}$ T26S (1-82)	(**oLM348** + oLM510) + (oLM509 + **oLM365**)[§]
pLM221	pKT25	$DnaA^{DI}$ N47A (1-82)	(**oLM348** + oLM512) + (oLM511 + **oLM365**)[§]
pLM246	pKT25	$DnaA^{DI}$ F49Y (1-82)	(**oLM348** + oLM547) + (oLM546 + **oLM365**)[§]
pLM247	pKT25	$DnaA^{DI}$ A50V (1-82)	(**oLM348** + oLM549) + (oLM548 + **oLM365**)[§]
pLM268	pKT25	$DnaA^{DI}$ T26M (1-82)	(**oLM348** + oLM607) + (oLM606 + **oLM365**)[§]
BsDnaD Plasmids			
pLM60	pKT25	$DnaD^{WHD}$ (1-128)	oLM148 + oLM176
pLM63	pUT18C	$DnaD^{FL}$ (1-232)	oLM180 + oLM181
pLM64	pUT18C	$DnaD^{WHD}$ (1-128)	oLM180 + oLM182
pLM65	pUT18C	$DnaD^{CTD}$ (129-232)	oLM183 + oLM181
pLM83	pKNT25	$DnaD^{CTD}$ (129-232)	oLM215 + oLM216
pLM113	pUT18C	$DnaD^{WHD}$ ΔNT_{ext} (19-128)	oLM308 + oLM182
pLM197	pUT18C	$DnaD^{WHD}$ F51A (1-128)	(**oLM180** + oLM361) + (oLM360 + **oLM182**)[§]
pLM198	pUT18C	$DnaD^{WHD}$ ΔLoop (1-128; Δ87-93 replaced with "GS")	(**oLM180** + oLM483) + (oLM482 + **oLM182**)[§]
pLM225	pUT18C	$DnaD^{WHD}$ I83A (1-128)	(**oLM180** + oLM520) + (oLM519 + **oLM182**)[§]
pLM224	pUT18C	$DnaD^{WHD}$ I76A (1-128)	(**oLM180** + oLM522) + (oLM521 + **oLM182**)[§]
pLM280	pKNT25	$DnaD^{WHD}$ (1-128)	oLM644 + oLM645
BsDnaB Plasmids			
pLM72	pKT25	$DnaB^{FL}$ (1-472)	oLM203 + oLM204
pLM120	pKNT25	$DnaB^{WHD}$ (1-153)	oLM327 + oLM328

Table S2 Continued

Plasmid	Vector	Description†	Primers‡
pLM151	pKNT25	$DnaB^{CTD1}$ (185-300)	oLM372 + oLM373
pLM153	pKNT25	$DnaB^{CTD2}$ (303-472)	oLM376 + oLM355
pLM277	pUT18C	$DnaB^{WHD}$ (1-153)	oLM634 + oLM635
oLM278	pUT18C	$DnaB^{CTD1}$ (185-300)	oLM636 + oLM637
oLM279	pUT18C	$DnaB^{CTD2}$ (303-472)	oLM638 + oLM639
BsDnaI Plasmids			
pLM266	pKNT25	$DnaI^{ZBD}$ (1-123)	oLM578 + oLM579
pLM267	pKNT25	$DnaI^{CTD}$ (124-311)	oLM582 + oLM583
pLM275	pUT18C	$DnaI^{ZBD}$ (1-123)	oLM640 + oLM641
pLM276	pUT18C	$DnaI^{CTD}$ (124-311)	oLM642 + oLM643
SaDnaA Plasmids			
pLM269	pKT25	$SaDnaA^{DI}$ (1-83)	oLM590 + oLM591
pLM270	pKT25	$SaDnaA^{DI}$ T25A (1-83)	(**oLM590** + oLM593) + (oLM592 + **oLM591**)§
pLM271	pKT25	$SaDnaA^{DI}$ F26A (1-83)	(**oLM590** + oLM595) + (oLM594 + **oLM591**)§
pLM272	pKT25	$SaDnaA^{DI}$ F48A (1-83)	(**oLM590** + oLM597) + (oLM596 + **oLM591**)§
SaDnaD Plasmids			
pLM273	pUT18C	$SaDnaD^{WHD}$ (1-124)	oLM600 + oLM601
EcDnaA Plasmids			
pLM274	pKT25	$EcDnaA^{DI}$ (1-90)	oLM602 + oLM603

†Amino acid range for each protein fragment is indicated in the brackets.
‡Primer pairs used to amplify the gene fragments are provided (see Table S1 for primer sequences).
§The primer extension method was used for site-directed mutagenesis. Primer pairs used in the first round of PCR are grouped together within the bracket, while the flanking primers used in the second round of PCR are in bold. See methods section for more details.

Table S3. Plasmids Used to Integrate *dnaD* Variants at the *amyE* Locus

Plasmid	Description	Primers[†]
pLM114	$DnaD^{FL}$	oLM309 + oLM310
pLM238	$DnaD^{FL}$ F51A	(**oLM309** + oLM361) + (oLM360 + **oLM310**)[‡]
pLM237	$DnaD^{FL}$ I83A	(**oLM309** + oLM520) + (oLM519 + **oLM310**)[‡]

[†]Primer pairs used to amplify the gene fragments are provided (see Table S1 for primer sequences).

[‡]The primer extension method was used for site-directed mutagenesis. Primer pairs used in the first round of PCR are grouped together within the bracket, while the flanking primers used in the second round of PCR are in bold. See methods section for more details.

Table S4. Plasmids Used for Protein Purifications

Plasmid	Description	Primers[†]
pLM2	$DnaD^{CTD}$ (129-232)	oLM5 + oLM6
pLM8	$DnaD^{WHD}$ (1-128)	oLM29 + oLM30
pLM45	$DnaB^{CTD2}$ (303-472)	oLM126 + oLM127
pLM47	$DnaB^{CTD1}$ (185-300)	oLM129 + oLM125
pLM165	$DnaA^{DI}$ (1-82)	oLM397 + oLM398
pLM175	$DnaD^{WHD}$ ΔNT (19-128)	oLM421 + oLM30
pLM176	$DnaB^{WHD}$ (1-153)	oLM422 + oLM423
pLM241	$DnaD^{WHD}$ F51A (1-128)	(**oLM29** + oLM361) + (oLM360 + **oLM30**)[‡]
pLM240	$DnaD^{WHD}$ I83A (1-128)	(**oLM29** + oLM520) + (oLM519 + **oLM30**)[‡]
pLM281	$DnaA^{DI}$ F49A (1-82)	(**oLM397** + oLM497) + (oLM496 + **oLM398**)[‡]

[†]Primer pairs used to amplify the gene fragments are provided (see Table S1 for primer sequences).

[‡]The primer extension method was used for site-directed mutagenesis. Primer pairs used in the first round of PCR are grouped together within the bracket, while the flanking primers used in the second round of PCR are in bold. See methods section for more details.

Table S5. *B. subtilis* strains used in this study

Strain Name	Genotype	Ectopically Expressed Protein	Source
PY79	Prototrophic	-	(Youngman, Perkins, & Losick, 1984)
LAM494.5	PY79 *dnaD23*	-	This study; mutation based on (Bruand, Sorokin, Serror, & Ehrlich, 1995)
LAM513	PY79 *dnaD23 amyE::P_{xyl}-dnaDF51A (erm)*	Full-length DnaD F51A	This study
LAM497	PY79 *dnaD23 amyE::P_{xyl}-dnaDI83A (erm)*	Full-length DnaD I83A	This study
LAM477	PY79 *dnaD23 amyE::P_{xyl}-dnaD (erm)*	Full-length DnaD	This study

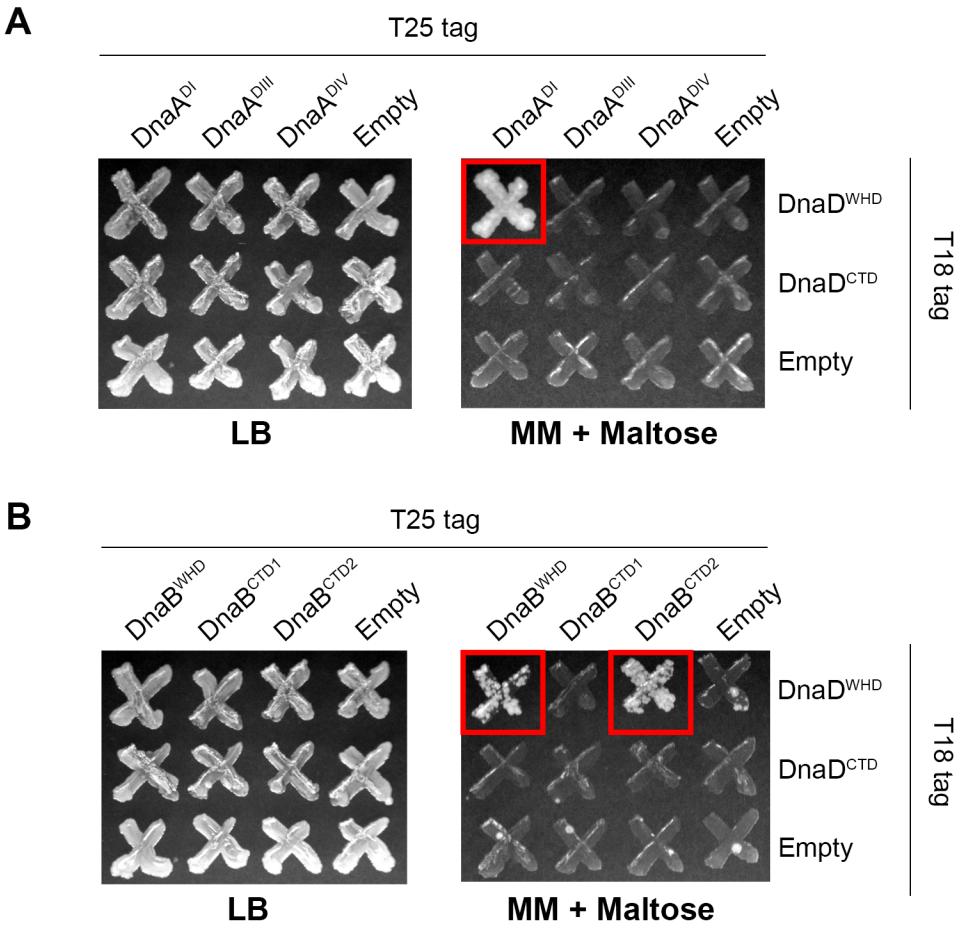

Figure S1. Detecting Interactions Between DnaA, DnaD and DnaB Using Minimal Media with Maltose.

B2H of T18-tagged DnaD domains co-expressed with T25-tagged DnaA domains **(A)** or T25-tagged DnaB domains **(B)** grown on LB or minimal media supplemented with 0.2% D-maltose (MM + Maltose). Red boxes indicate positive interactions.

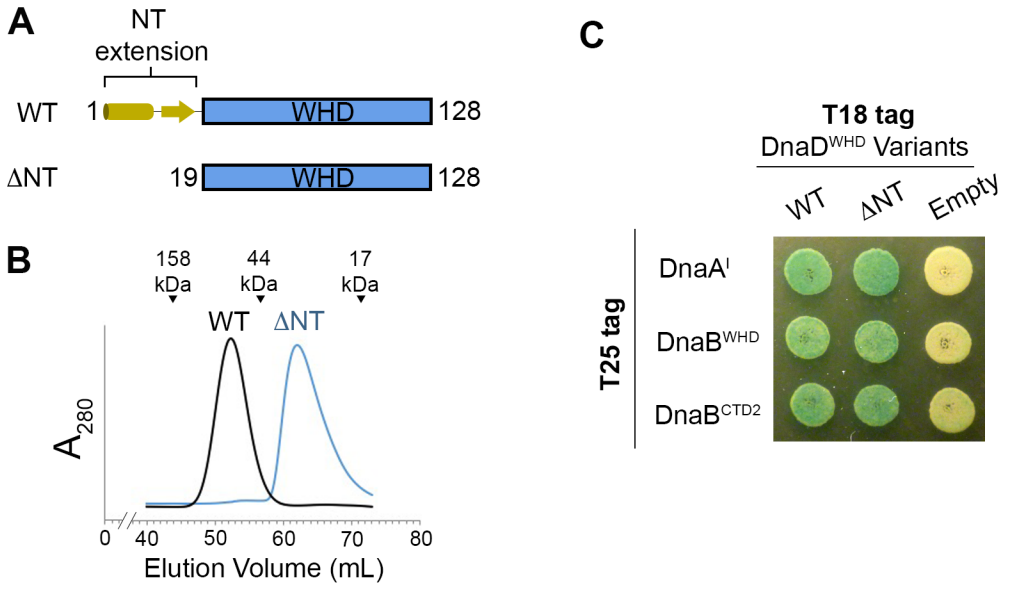

Figure S2. The DnaD^{WHD} NT Extension is Not Necessary for Protein Interactions.

(A) Schematic of DnaD^{WHD} showing the N-terminal extension (residues 1-18) that mediates tetramerization. **(B)** Size exclusion chromatogram of either wild type DnaD^{WHD} (black) or the ΔNT variant (blue) with elution volume (mL) labeled on the x-axis and absorbance at 280 nm (A_{280}) labeled on the y-axis. The ΔNT variant elutes later than expected for the tetramer (size of the ΔNT tetramer is 51.9 kDa) demonstrating that this truncation has shifted the equilibrium to favor the dimer. **(C)** B2H of T18-tagged wild type DnaD^{WHD} or the ΔNT variant co-expressed with T25-tagged DnaA^{DI}, DnaB^{WHD} or DnaB^{CTD2}.

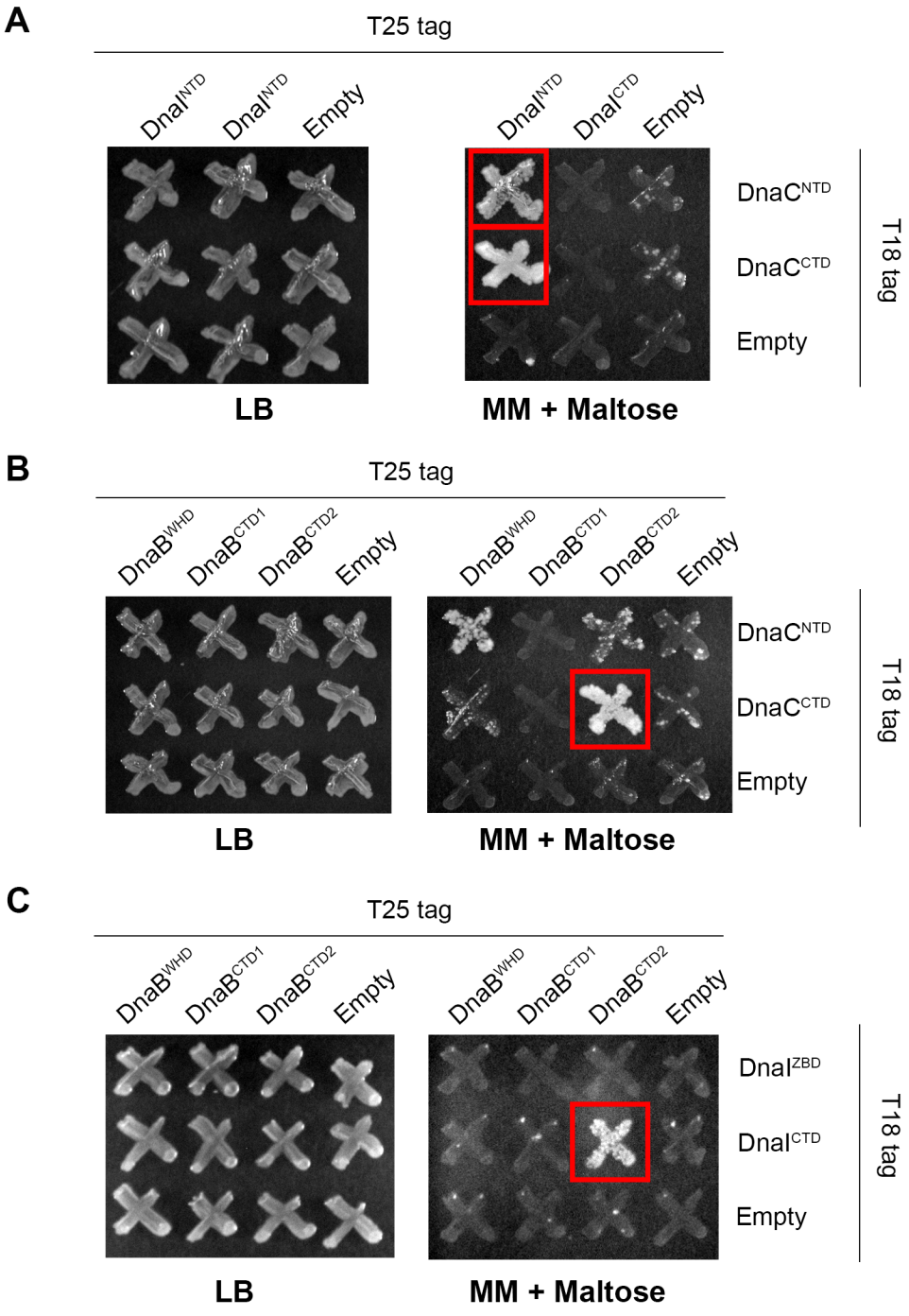

Figure S3. Detecting Interactions with the DnaC Helicase Using Minimal Media with Maltose.

Figure S3 continued

(A) B2H of T18-tagged DnaC domains co-expressed with T25-tagged DnaI domains. **(B)** B2H of T18-tagged DnaC domains co-expressed with T25-tagged DnaB domains. **(C)** B2H of T25-tagged DnaB domains co-expressed with T18-tagged DnaI domains. The cells were grown on LB or minimal media supplemented with 0.2% D-maltose (MM + Maltose). Red boxes indicate positive interactions.

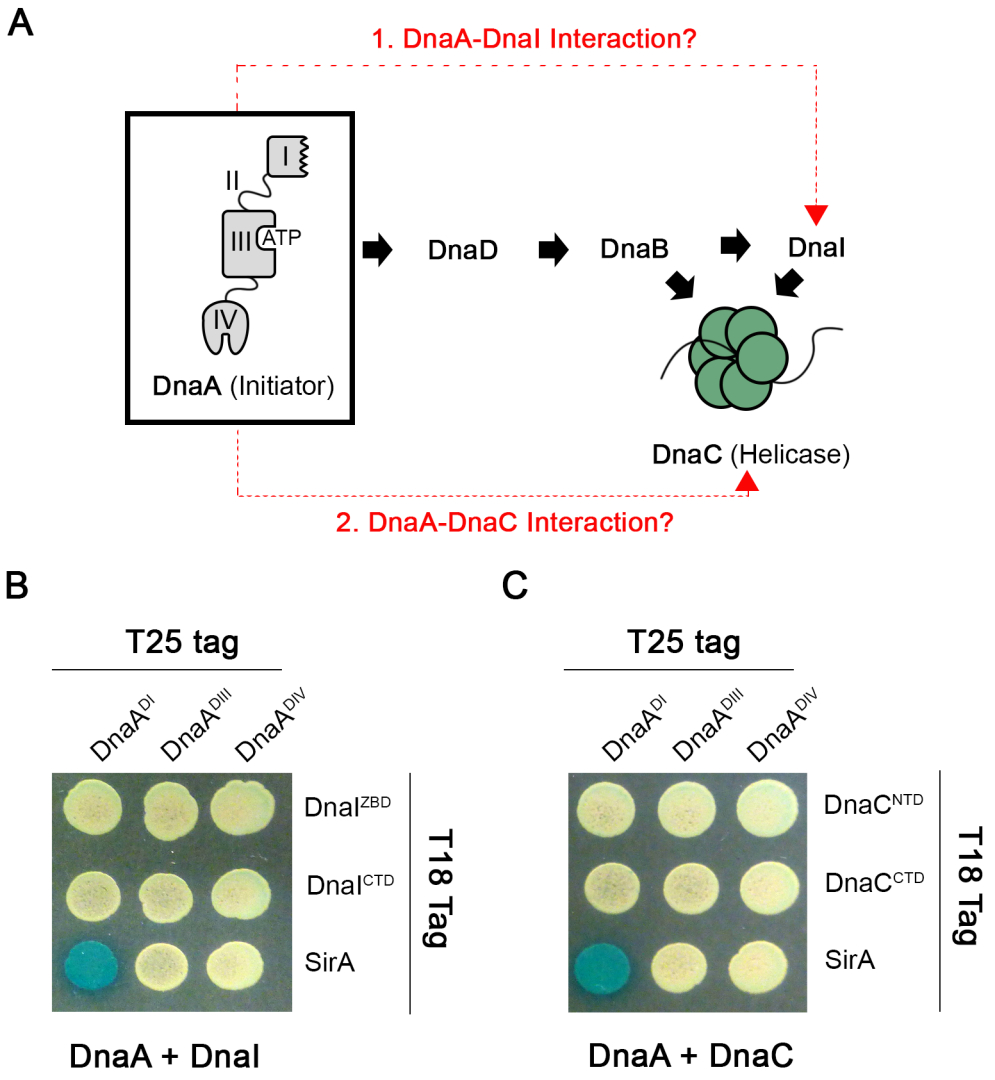

Figure S4. *B. subtilis* DnaA Cannot Bind DnaI or DnaC.

(A) Schematic of the *B. subtilis* helicase loading pathway with red arrows indicating the interactions tested in the B2H assay and black arrows indicating known protein interactions. **(B)** B2H of T25-tagged DnaA domains co-expressed with T18-tagged DnaI domains. **(C)** B2H of T25-tagged DnaA domains co-expressed with T18-tagged DnaC domains. SirA is included as a positive control.

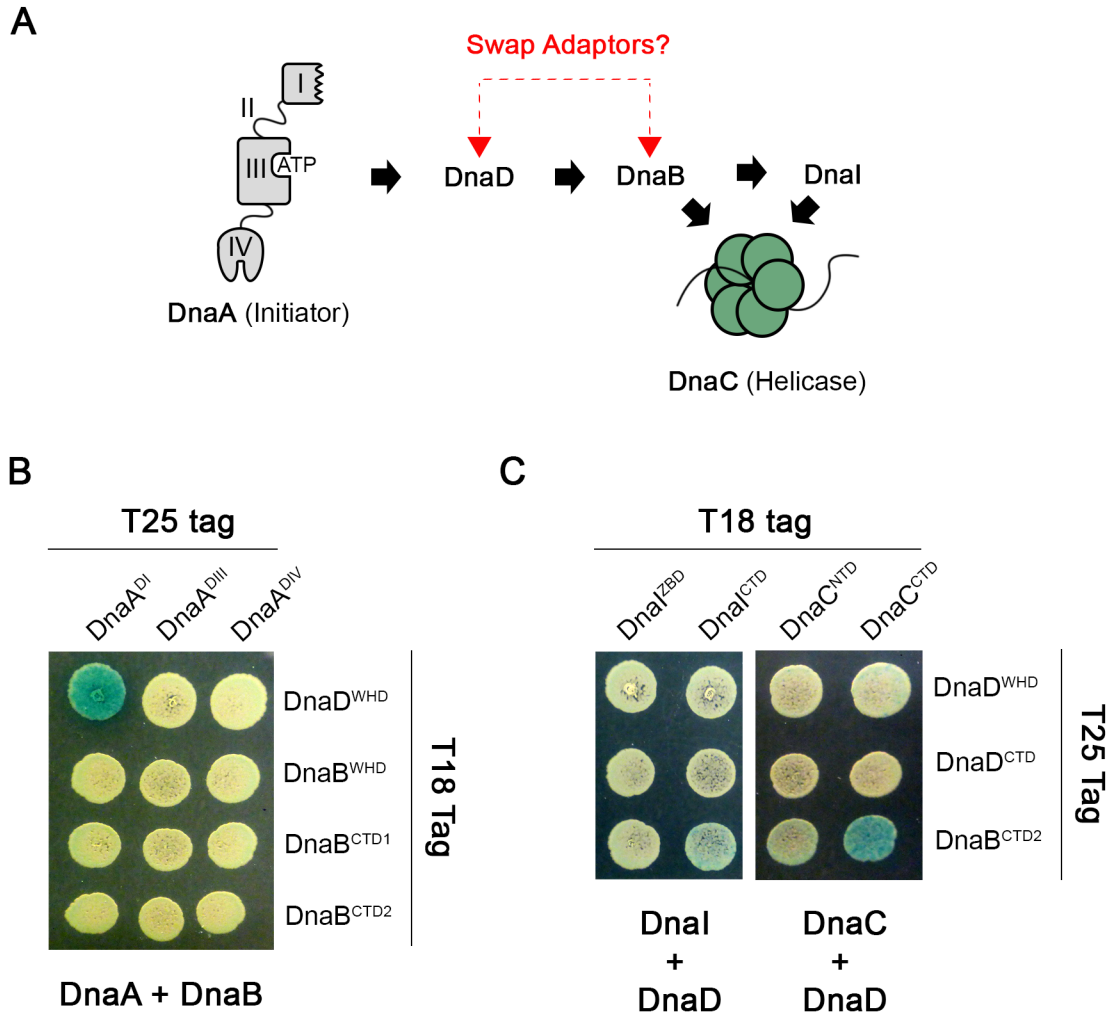

Figure S5. DnaD and DnaB Cannot Substitute for Each Other in the B2H Assay.

(A) Schematic of the *B. subtilis* helicase loading pathway with known interactions indicated by black arrows. **(B)** B2H of T25-tagged DnaA domains co-expressed with T18-tagged DnaB domains. The DnaD^{WHD} domain is included as a positive control. **(C)** B2H of T18-tagged DnaI domains (left) or DnaC domains (right) co-expressed with T25-tagged DnaD domains. The DnaB^{CTD2} domain is included as a positive control.

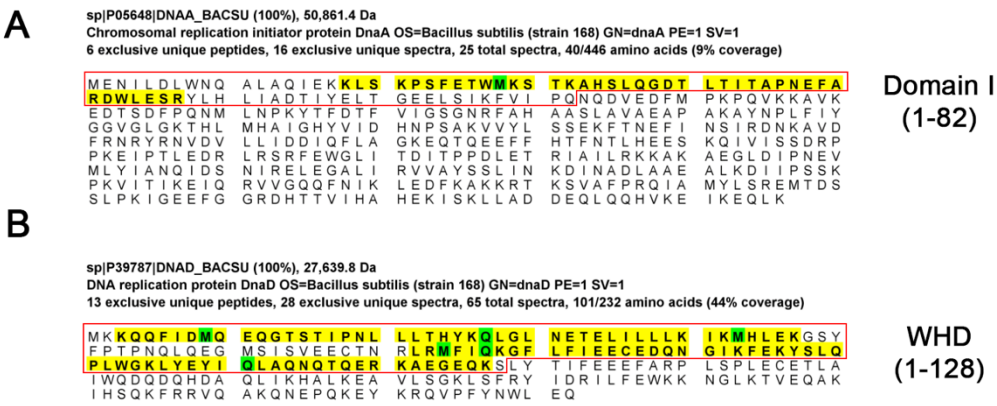

Figure S6. Mass Spectrometry Results for the DnaD^{WHD}-DnaA^{DI} Crosslinked Band. Both DnaA **(A)** and DnaD **(B)** were detected in the crosslinked band. Identified peptides are highlighted in yellow with the boundaries of the DnaD^{WHD} and DnaA^{DI} domains marked with red lines. Note that only the isolated DnaD^{WHD} and DnaA^{DI} were used in the crosslinking experiment. The mass spectrometry was performed by MS Bioworks (Ann Arbor, MI, www.msbioworks.com).

References

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