

Supplemental material

Table S4.1. List of bacterial strains used in this study.

Strain	Characteristics	Source
<i>E. coli</i> MG1655 (wild type)	K-12; F ⁻ λ : <i>rph-1</i>	ATCC
<i>E. coli</i> Δ 3D	MG1655; <i>strR</i> Δ DARS1 Δ DARS2 Δ datA	318
<i>E. coli</i> DH5 α	MG1655; <i>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG</i> ϕ 80d <i>lacZ</i> Δ M15 Δ (<i>lacZYA-argF</i>)U169 <i>hsdR17</i> (r _K ⁻ m _K ⁺)	NEB
<i>E. coli</i> DH5 α x pCas9	DH5 α harbouring the pCas9 plasmid	This study
<i>E. coli</i> DH5 α x pCas9_ampR	DH5 α harbouring the pCas9_ampR plasmid	This study
<i>E. coli</i> DH5 α x pTarget_dnaA-PAFP	DH5 α harbouring one of the 9 plasmids of the pTarget_dnaA-PAFP series	This study
<i>E. coli</i> MG1655 x pCas9, pTarget_dnaA-PAFP	MG1655 harbouring the pCas9 plasmid and one of the ten plasmids of the pTarget_dnaA-PAFP series	This study
<i>E. coli</i> MG1655 <i>dnaA-PAFP</i> x pCas9, pTarget_dnaA-PAFP	MG1655 harbouring the pCas9 plasmid and one of the ten plasmids of the pTarget_dnaA-PAFP series; <i>dnaA</i> Δ (259-312)::PAFP	This study
<i>E. coli</i> <i>dnaA-PAmCherry2.1</i>	MG1655; <i>dnaA</i> Δ (259-312)::PAmCherry2.1	This study
<i>E. coli</i> <i>dnaA-dronpa2</i>	MG1655; <i>dnaA</i> Δ (259-312)::dronpa2	This study
<i>E. coli</i> <i>dnaA-mEos4b</i>	MG1655; <i>dnaA</i> Δ (259-312)::mEos4b	This study
<i>E. coli</i> <i>dnaA-mMaple3</i>	MG1655; <i>dnaA</i> Δ (259-312)::mMaple3	This study
<i>E. coli</i> Δ 3D x pCas9_ampR, pTarget_dnaA-PAmCherry2.1	MG1655 harbouring the pCas9_ampR and the pTarget_dnaA-PAmCherry2.1 plasmids; <i>strR</i> Δ DARS1 Δ DARS2 Δ datA	This study
<i>E. coli</i> Δ 3D <i>dnaA-PAmCherry2.1</i> x pCas9_ampR, pTarget_dnaA-PAmCherry2.1	MG1655 harbouring the pCas9_ampR and the pTarget_dnaA-PAmCherry2.1 plasmids; <i>strR</i> Δ DARS1 Δ DARS2 Δ datA <i>dnaA</i> Δ (259-312)::PAmCherry2.1	This study
<i>E. coli</i> Δ 3D <i>dnaA-PAmCherry2.1</i>	MG1655; <i>strR</i> Δ DARS1 Δ DARS2 Δ datA <i>dnaA</i> Δ (259-312)::PAmCherry2.1	This study

Table S4.2. List of plasmids used in this study. *DnaA*(*n1-n2*) stands for the sequence of the *dnaA* gene comprised between base pair *n1* and base pair *n2*, counting from the G at position 1.

Plasmid	Characteristics	Source
pCas9	<i>repA101</i> (Ts) <i>kanR</i> <i>cas9</i> <i>P</i> _{araB-Red} <i>lacI</i> ^q <i>P</i> _{trc} -sgRNA-pMB1	319
pCas9_ampR	pCas9; Δ <i>kanR</i> <i>ampR</i>	This study
pTarget	pMB1; <i>aadA</i> <i>pJ23119</i>	319
pTarget_dnaA-PAFP series		
pTarget_dnaA-PAmCherry2.1	pTarget; <i>pJ23119</i> -sgRNA(<i>dnaA</i>) <i>dnaA</i> (209-258)-PAmCherry2- <i>dnaA</i> (313-362)	This study
pTarget_dnaA-Dronpa2	pTarget; <i>pJ23119</i> -sgRNA(<i>dnaA</i>) <i>dnaA</i> (209-258)-Dronpa2- <i>dnaA</i> (313-362)	This study
pTarget_dnaA-mEos4b	pTarget; <i>pJ23119</i> -sgRNA(<i>dnaA</i>) <i>dnaA</i> (209-258)-mEos4b- <i>dnaA</i> (313-362)	This study
pTarget_dnaA-mMaple3	pTarget; <i>pJ23119</i> -sgRNA(<i>dnaA</i>) <i>dnaA</i> (209-258)-mMaple3- <i>dnaA</i> (313-362)	This study

Table S4.3. List of oligonucleotides and chemically synthesised DNA fragments and their use in the study.

Identifier	Sequence (5'-3')	Used for
BG25452	ATGTCGGTGATCAAACCAGATATGAAGATCAAACCTTCGTATGGAGGGAGCGGT CAATGGGCATCCTTTTCGCGATCGAGGGTGTGCGGGCTGGGCAAACCCTTCGAA GGGAAGCAAAGTATGGACTTAAAAGTCAAAGAGGGAGGCCCGTTACCCTTTG CGTATGATATTTTGACCACAGTTTTTGTACGGGAATCGCGTATTTGCTAAGTA CCCGGAAAACATCGTCGATTACTTCAAACAGAGCTTCCCAGAAGGATATAGTT GGGAGCGCTCAATGAACACGAGGACGGTGGCATTGTGAATGCGACGAACGA CATTACATTAGACGGCGACTGCTACATTTATGAGATCCGTTTCGACGGTGTCAA CTTTCCAGCGAATGGACCTGTGATGCAAAAGCGCACTGTAAAATGGGAACCTT CAACCGAGAAGTTATATGTGCGTGACGGGGTCTTAAAAGGCGATGTAAATACC GCTTTGAGTTTAGAGGGAGGTGGCCATTACCGTTGCGATTTCAAACGACTTAT AAGGCAAAAAAGTGGTACAATTACCTGATTATCACTTCGTCGATCATCACATC GAGATTAAATCACATGATAAGGACTATTCTAATGTGAATCTTCACGAACATGCC GAGGCCCATAGTGAGTTACCGCGTCAAGCAAAGTAA	<i>E. coli</i> codon- optimised <i>dronpa2</i> .
BG28278	ATGGTTAGTGCGATTAAACCAGATATGCGTATCAAGTTGCGTATGGAGGGAAA TGTTAATGGACATCACTTCGTTCATTGATGGAGACGGAAGTGGCAAGCCGTACG AGGGTAAGCAGACCATGGACCTGGAGGTCAAGGAGGGTGGCCCATGCGCTT CGCGTTTGATATCCTGACGACAGCTTTCCACTATGGGAATCGTGTCTTCGTAAA ATATCCAGATAACATCCAGGACTATTTCAAGCAGTCATTTCCCAAAGGTTACTCT TGGAACGCAGCTTAACCTTCGAAGACGGGGGGATTGCAACGCCCGCAACG ACATTACTATGGAGGGAGACACGTTTTACAACAAAGTGCCTTTTTATGGAACAA ACTTCCCGGCGAACGGGCCTGTTATGCAGAAGAAGACTCTGAAGTGGGAGCC GTCCACGGAAAAGATGTACGTGCGCGATGGGGTCTTAAGTGGGGATATTGAG ATGGCCTTGCTGCTTGAAGGTAATGCTCACTACCGCTGCGATTTCCGTACGACC TATAAGGCAAAAAGAAAAGGGGGTCAAGTTGCCAGGTGCTCATTTTGTGATCA CGCGATTGAAATTTGTGCGATGATAAGGATTACAATAAGGTTAACTTTACGA GCATGCGGTGGCTCATAGCGGTCTTCCCGACAATGCCCGTCGTAA	<i>E. coli</i> codon- optimised <i>mEos4b</i> .
BG30037	ATGGTCTCTAAGGGAGAAGAGACGATCATGTGCGTAATCAAACCGGATATGAA AATCAAGCTTCGCATGGAAGGTAACGTCAACGGTCACGCCTTTGTATCGAAG GTGAAGGCTCAGGTAAGCCATTTGAAGGTATCCAAACCATCGACTTGGAAGTA AAGGAGGGTGCGCTTTACCATTCGCATACGACATTTTACCACCGCTTTCCAT TACGGAAACCGCGTGTTACCAAGTACCTTCGCAAGATCCCTGACTACTTCAAA CAGAGCTTCCCAGAGGGATATTCTTGGAACGTAGTATGACGTACGAGGACG GGGTATCTGCAATGCGACTAATGATATTACAATGGAAGAAGATTCGTTTATCA ATAAAATTCACCTCAAAGGTACGAATTTCCGCCAACGGCCCGGTAATGCAGA AACGTACTGTAGGTTGGGAGGTCTCGACTGAAAAGATGTATGTTCTGTGACGGC GTGCTGAAGGGAGATGTTAAATGAAGCTGCTTCTTAAGGGTGGCTCCATTAT CGCTGTGATTTTCGTACCACATACAAGGTGAAGCAAAAAGCAGTGAAATTGCC CAAAGCACACTTTGTGACCATCGTATCGAGATCCTTTCTCACGATAAAGATTA CAACAAGGTTAAGTTGTATGAACATGCTGTAGCTCGCAATTCCACAGATAGTAT GGACGAGCTGTATAAATAA	<i>E. coli</i> codon- optimised <i>mMaple3</i> .
BG21364	TCAGCCTTAGTCATTATCGAC	Amplified the <i>dnaA</i> gene in <i>E.</i> <i>coli</i> chromosome
BG21365	GGTTACGATGACAATGTTCTG	Amplified the <i>dnaA</i> gene in <i>E.</i> <i>coli</i> chromosome

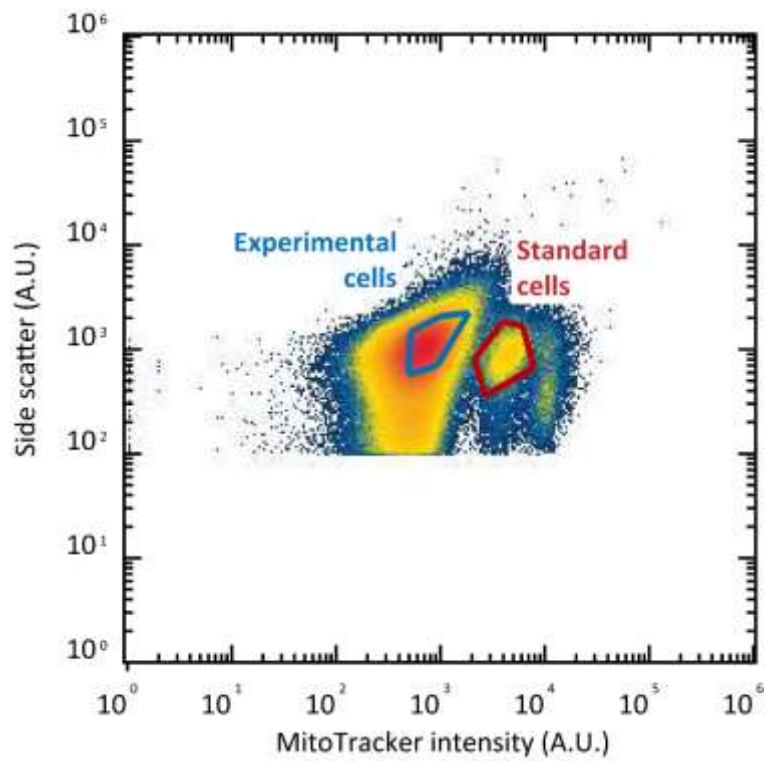


Figure S4.1. Separation of standard and experimental cell by YL3-H gating. Cells with a higher red emission, derived from the MitoTracker™ dye, were gated as standard cells, whereas the other population was gated as experimental cells.

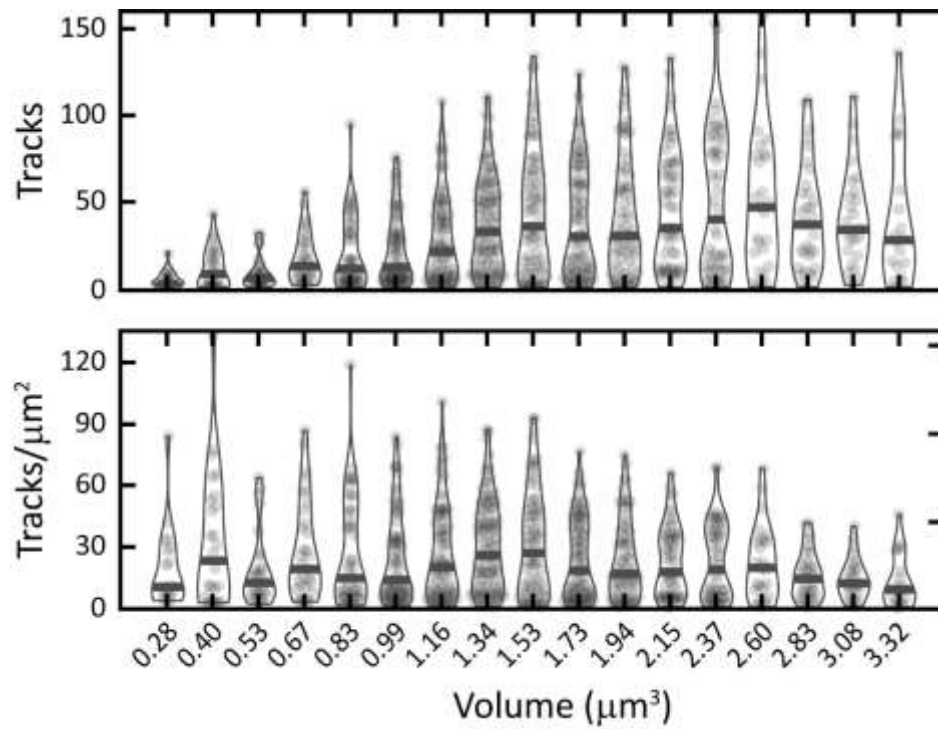


Figure S4.2. Tracks and concentration of *LbdCas12a* under the control of simple repression, mediated by the *pLtetO-1* promoter and the related TetR repressor. On the contrary of negative autoregulation, simple repression causes accumulation of new protein only until a certain level. After this level, the track count stabilises and the concentration decreases.

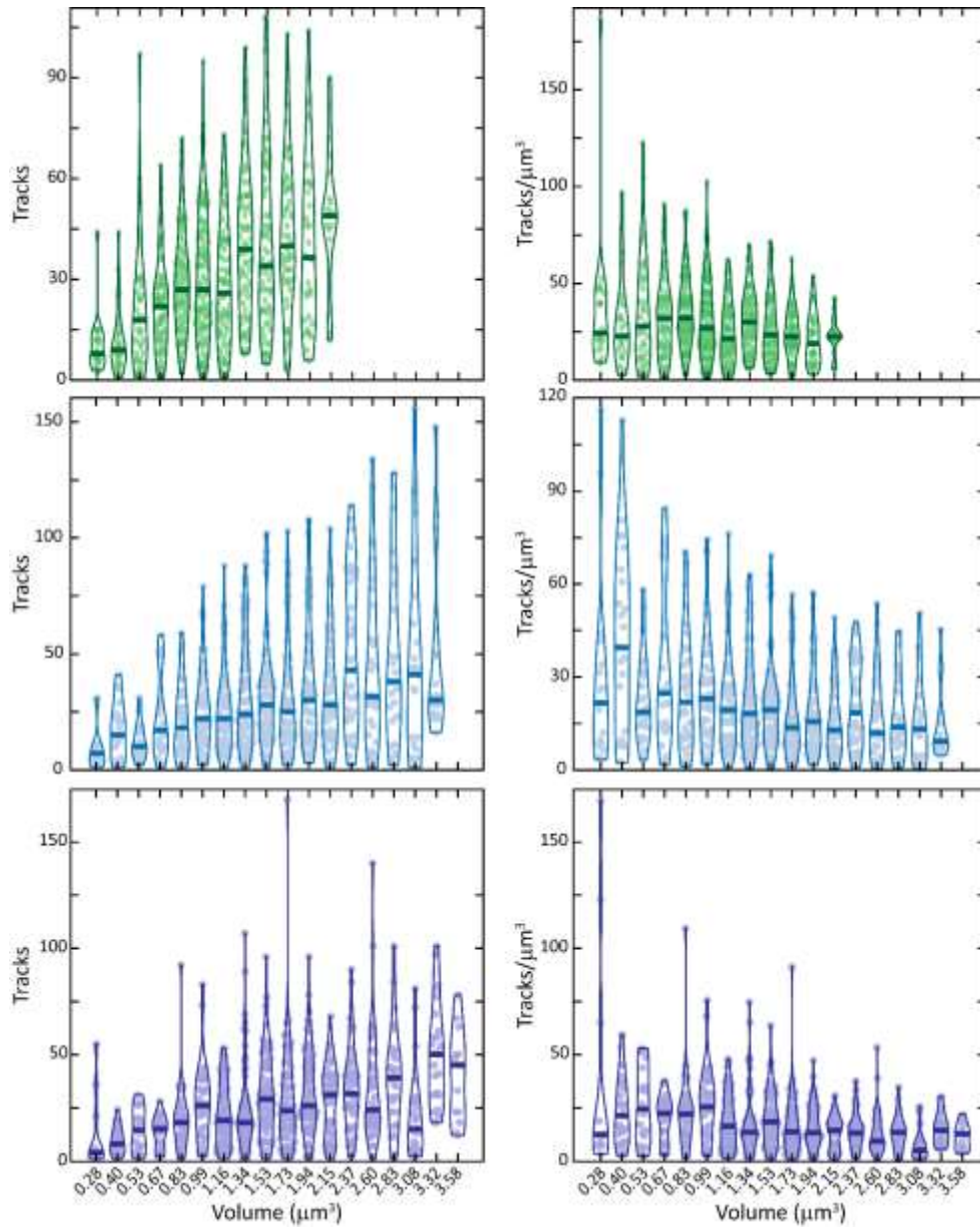


Figure S4.3. Track number and concentration for DnaA in *E. coli* Δ3D. For each growth condition and cellular volume range, the number of recorded tracks per cell (left) and the concentrations of recorded tracks per cell (tracks/μm³, right) are plotted as violin plots. On the x-axis, the number indicates the middle value of the range. Dots represent individual values of cells in the indicated volume range, whereas bold lines represent the median. Green indicates values of cells grown in slow growth regime, blue values of cells grown in intermediate growth regime and purple values of cells grown in fast growth regime.

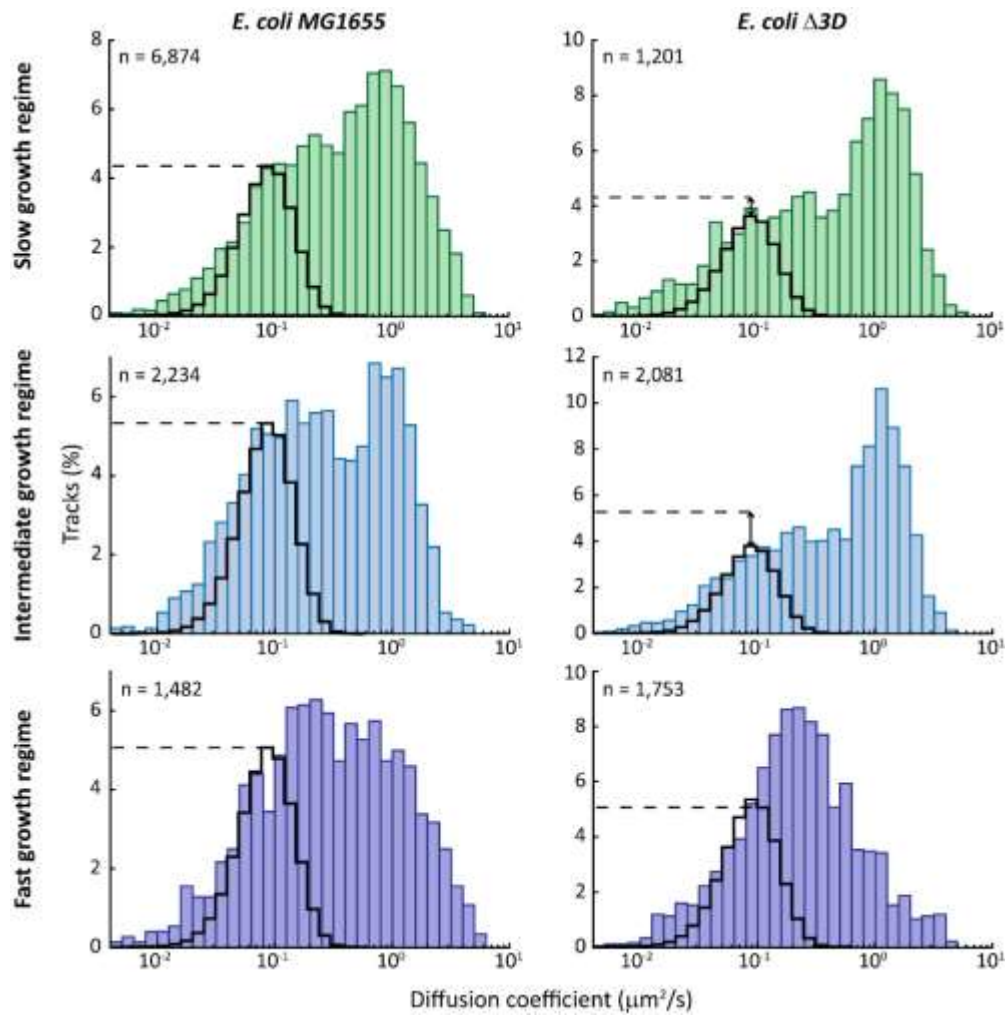


Figure S4.4. Persistent binding of DnaA across growth conditions and *E. coli* genotype. Diffusional coefficients of DnaA tracks longer than 5 steps (> 6 localisations, > 50 ms long), collected into 36 logarithmic-divided bins from $D^* = 0.04 \mu\text{m}^2/\text{s}$ to $D^* = 10 \mu\text{m}^2/\text{s}$. Green histograms collect tracks derived from either *E. coli* MG1655 or *E. coli* $\Delta 3D$ cells grown in slow growth regime. Blue histograms collect tracks derived from either *E. coli* MG1655 or *E. coli* $\Delta 3D$ cells grown in intermediate growth regime. Purple histograms collect tracks derived from either *E. coli* MG1655 or *E. coli* $\Delta 3D$ cells grown in fast growth regime. Black lines represent an hypothetical immobile and static species with a track length of 5 and was used as a qualitative measure for persistent binding in each condition. The dashed line represent the percentage of DnaA tracks in the wild type genotype at the peak of the hypothetical immobile species. The line was used as a comparison between the two strains to qualitative assess a reduction in persistent binding upon deletion of *DARS1*, *DARS2* and *datA*.

Source data

Source Data Table 4.1. Source data of kinetic rates of DnaA in either slow, intermediate or fast growth regime, used for the scatter plot in Figure 4.2C.

Growth regime	$k_{\text{Free} \rightarrow \text{bound}} (\text{S}^{-1})$		$k_{\text{Bound} \rightarrow \text{free}} (\text{S}^{-1})$	
	Value	St. dev.	Value	St. dev.
Slow	110	5	46	4
Intermediate	107	5	57	3
Fast	95	9	75	7

Source Data Table 4.2. Source data of kinetic rates of DnaA in the wild type *E. coli* MG1655, in either slow, intermediate or fast growth regime, used for the scatter plot in Figure 4.2C.

Growth regime	Bound fraction (%)	St. dev.
Slow	70.5	4.3
Intermediate	65.2	3.8
Fast	55.8	6.5

Source Data Table 4.3. Extremes of each volume range used for the violin plots in Figure 4.3 and Figure 4.4D.

Pixel range	Volume range (μm^3)	Middle point (μm^3)
50-65	0.23-0.34	0.28
66-80	0.34-0.46	0.40
81-95	0.46-0.6	0.53
96-110	0.6-0.75	0.67
111-125	0.75-0.9	0.83
126-140	0.9-1.07	0.99
141-155	1.07-1.25	1.16
156-170	1.25-1.44	1.34
171-185	1.44-1.64	1.53
186-200	1.64-1.83	1.73
201-215	1.83-2.04	1.94
216-230	2.04-2.26	2.15
231-245	2.26-2.46	2.37
246-260	2.46-2.71	2.60
261-275	2.71-2.95	2.83
276-290	2.95-3.2	3.08
291-305	3.2-3.45	3.32
306-320	3.45-3.71	3.58

Source Data Table 4.4. Source data of kinetic rates and derived bound fractions of DnaA in the mutant *E. coli* $\Delta 3D$, in either slow, intermediate or fast growth regime, used for the bar plot of bound fractions in Figure 4.4E.

Regime	$k_{\text{Free} \rightarrow \text{Bound}}$		$k_{\text{Bound} \rightarrow \text{Free}}$		Bound fraction	
	Value	St. dev.	Value	St. dev.	Value	St. dev.
Slow	80	5	58	4	0.57971	0.045125
Intermediate	109	5	63	7	0.633721	0.043007
Fast	116	7	68	8	0.630435	0.052667

Source Data Table 4.5. Source data of volumes of initiation of either the wild type *E. coli* MG1655 or the mutant *E. coli* $\Delta 3D$, in either slow, intermediate or fast growth regime, used for the bar plot in Figure 4.4F.

Strain	Growth regime	Volume initiation	St. dev.
<i>E. coli</i> MG1655	Slow	0.527963242	0.128615451
	Intermediate	0.366663738	0.068312876
	Fast	0.377714632	0.032840001
<i>E. coli</i> $\Delta 3D$	Slow	0.606818985	0.07243655
	Intermediate	0.686966969	0.057704679
	Fast	0.534894582	0.050480645

Source Data Table 4.6. Cellular volume and number of origins of either *E. coli* MG1655 or *E. coli* $\Delta 3D$ in either slow, intermediate or fast growth regime, used to estimate the volume of initiation plotted in the bar graph of Figure 4.4F.

Strain	Growth regime	Average volume (μm^3)		Number of origins	
		Value	St. dev.	Value	St. dev.
<i>E. coli</i> MG1655	Slow	0.678465868	0.16522444	1.853953582	0.011595011
	Intermediate	1.010858586	0.187395417	3.977379052	0.073825525
	Fast	1.286390533	0.107618857	4.91341633	0.116306801
<i>E. coli</i> $\Delta 3D$	Slow	1.217928	0.144470366	2.895589507	0.038714931
	Intermediate	1.846496063	0.154868456	3.877814851	0.017952345
	Fast	1.797699029	0.132968393	4.848677779	0.284203588