

Supplemental material

Table S5.1. List of bacterial strains used in this study.

Strains	Characteristics	Source
<i>E. coli</i> MG1655 (wild type)	K-12; F ⁻ λ^- <i>rph-1</i>	ATCC
<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> (<i>r_K</i> ⁻ <i>m_K</i> ⁺)	NEB
<i>E. coli</i> DH5 α x pUC19_cat-pLtetO-1-TetR	DH5 α harbouring the pUC19_cat-pLtetO-1-TetR plasmid.	This study
<i>E. coli</i> DH5 α x pUC19_dnaAcos-KanR	DH5 α harbouring the pUC19_dnaAcos-KanR plasmid	This study
<i>E. coli</i> DH5 α x pSC020	DH5 α harbouring the pSC020 plasmid	This study
<i>E. coli</i> MG1655 x pSC020	MG1655 harbouring the pSC020 plasmid	This study
<i>E. coli</i> pre-CoRTeDcos x pSC020	MG1655 harbouring the pSC020 plasmid; Δ <i>DnaAp2</i> , Δ <i>dnaA::lox66-cmR-lox71-pLtetO-1-tetR-dnaAcos-lox66-kanR-lox71</i>	This study
<i>E. coli</i> CoRTeDcos x pSC020	MG1655 harbouring the pSC020 plasmid; Δ <i>DnaAp2</i> , Δ <i>dnaA::lox-pLtetO-1-tetR-dnaAcos-lox</i>	This study
<i>E. coli</i> CoRTeDcos	MG1655; Δ <i>DnaAp2</i> , Δ <i>dnaA::lox-pLtetO-1-tetR-dnaAcos-lox</i>	This study
<i>E. coli</i> CoRTeDcos-R2G	MG1655; Δ <i>DnaAp2</i> , Δ <i>dnaA::pLtetO-1-tetR-dnaAcos</i> ; A-to-G mutation in box R2 of <i>oriC</i>	This study

Table S5.2. List of plasmids used in this study.

Plasmid	Characteristics	Source
pSC020	<i>repA101, oriV, ampR, araC, P_{araB}-Red, lacI^q, P_{Lac}-Cre</i>	353
pUC19_cat-pLtetO-1-TetR	pUC19, <i>ampR, lox66-cmR-lox71, pLtetO-1-tetR</i>	This study
pUC19_dnaAcos-kanR	pUC19, <i>ampR, dnaAcos, lox66-kanR-lox71</i>	This study

Table S5.3. List of oligonucleotides and DNA fragments mentioned in the study. Nucleotides in bold contained the mentioned features of interest.

Identifier	Sequence (5'-3')	Used for
BG22166	cgagtggagtcgcca atgtcactttcgctttggcagcagtgcttggccgattgcagg atgagttaccagccacagaattcagtatgtggatacgccattgcaggcggaactg agcgataacacgctggccctgtacgcgccaacacgttttgtcctcgattgggtacgg gacaagtaccttaataatatcaatggactgtaaccagtttctgcggagcggatgcc ccacagctgcgttttgaagtggcaccacacacggtgacgcaaacgccacaagcgg cagtgacgagcaacgtcgcgccccctgcacaggtggcgcaaacgcagccgcaac gtgtgcgccttctacgcgctcaggttgggataacgtcccggccccgcgagaaccg acctatcggttctaacgtaaacgtcaaacacacggttgataacttcgttgaaggtaat ctaaccaactggcgcgcgcgcggtcgccTTgtggcgataaacctggcggtgc ctataacccgttgttcctttatggcggcacgggtctgggtaaaactcacctgctgcat gTTgtgggtaacggcattatggcgcgcaagccgaatgccaaagtggtttatatgc actccgagcgctttgttcaggacatggttaagccctgcaaaacaacgcgatcgaa gagtttaaacgctactaccggttcgtagatgcactgctgatcgacgatattcagttttt tgctaataaagaacgatctcaggaagagttttcTATacctcaacgccctgctgga aggtaataacagatcattctcacctcgatcgccCACccgaagagatcaacggc gttgaggatcggttgaaatcccgttcggttggggactgactgtggcgatcgaaccg ccagagctggaaacccgtgtggcgatcctgatgaaaaggccgacgaaaacga cattcgtttgcggggcgaaagtggcggttctttatcgccaagcgctacgatctaactga cgtgagctggaaggggcgctgaaccgcgtcattgccaatgccaactttaccggac ggcgatcaccatcgacttcgtgcgtgagggcgctgcgcgacttgcgtggcattgcag gaaaaactggtcaccatcgacaatatcagaagacggtggcggagtactacaag atcaaagtcgcgatctcctttccaagcgatcccgtcggtggcgctccgcgc cagatggcgatggcgctggcgaaagagctgactaaccacagctgccggagatt ggcgatgcgtttgggtggccgtgaccacacgacggtgcttcattgcctgccgtaagatc gagcagttgcgtgaagagagccacgatataaagaagattttcaaatttaacgag aacattgtcatcgtaaacctat	Synthetic DNA of <i>dnaAcos</i>
BG22660	gcctcgcggcaggatcggttacacttagcagagttctggaaagtcctgtggg ctagtt attgctcagcggtgg	Introduced upstream homology arm to the <i>lox66-cat-lox71-pL tetO-1-tetR-BsaI</i> fragment.
BG20764	gcgtttcggtgatgacggtgaaaac	Amplified the <i>lox66-cat-lox71-pL tetO-1-tetR-BsaI</i> fragment, together with BG22660.
BG21406	cgattcattaatgcagctggcacg	Amplified the <i>BsaI-dnaAcos-lox66-kanR-lox71</i> fragment, together with BG22661.
BG22661	taaatgctcacggttctacggtaaat ttcataggtttacgatgacaatggtccgactgg aaagctaccg	Introduced downstream homology arm to <i>dnaAcos-lox66-kanR-lox71</i> fragment.
BG22467	cggcaatttcgcgccttc	Used for colony PCR and Sanger sequencing of the <i>dnaA</i> locus.
BG22496	gacctcacaccagtggaaaccag	Used for colony PCR and Sanger sequencing of the <i>dnaA</i> locus.

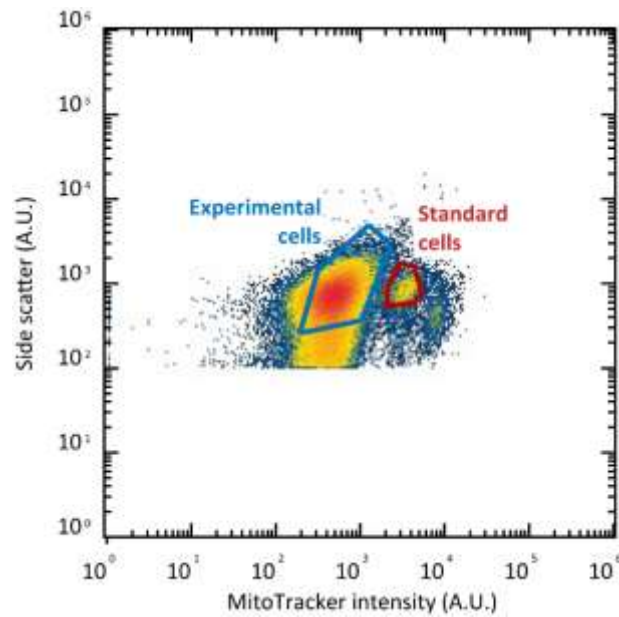


Figure S5.1. Separation of standard and experimental cell by MitoTracker™ intensity. Cells with a higher red emission, derived from the MitoTracker™ dye, were gated as standard cells, whereas the other population was gated as experimental cells.

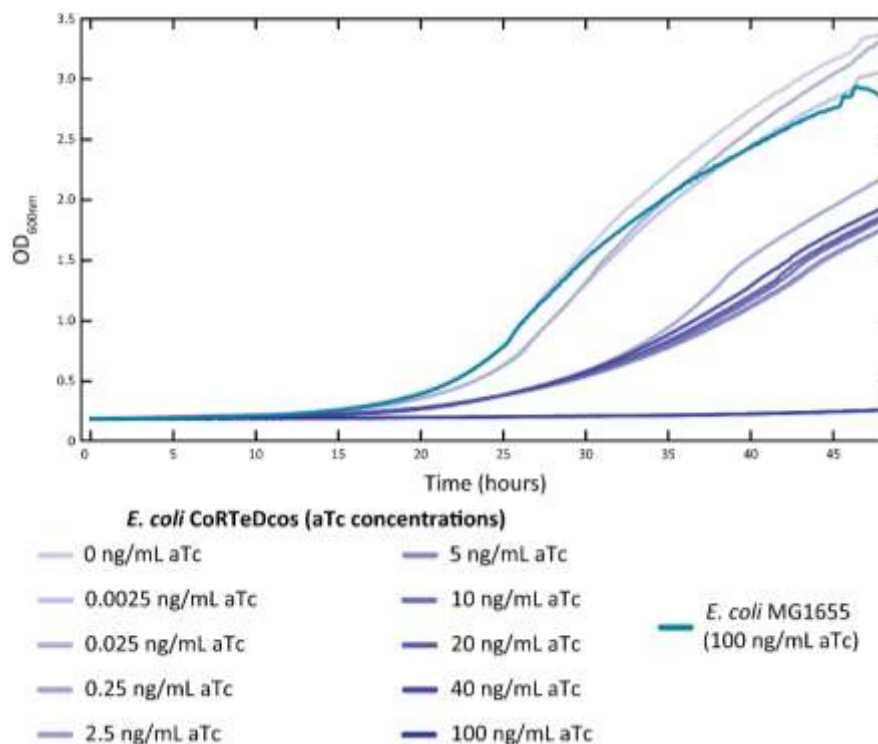


Figure S5.2. Growth curves of *E. coli* CoRTEdcos in slow growth regime in different concentrations of aTc. The growth curve related to the wild-type is in presence of 100 ng/mL.

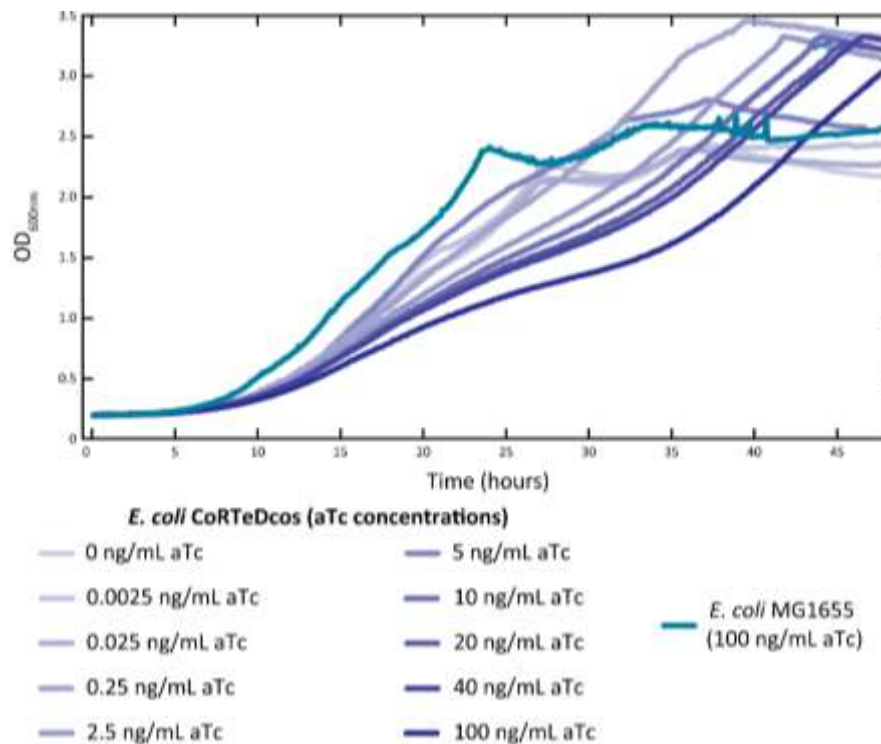


Figure S5.3. Growth curves of *E. coli* CoRteDcos in intermediate growth regime in different concentrations of aTc. The growth curve related to the wild-type is in presence of 100 ng/mL.

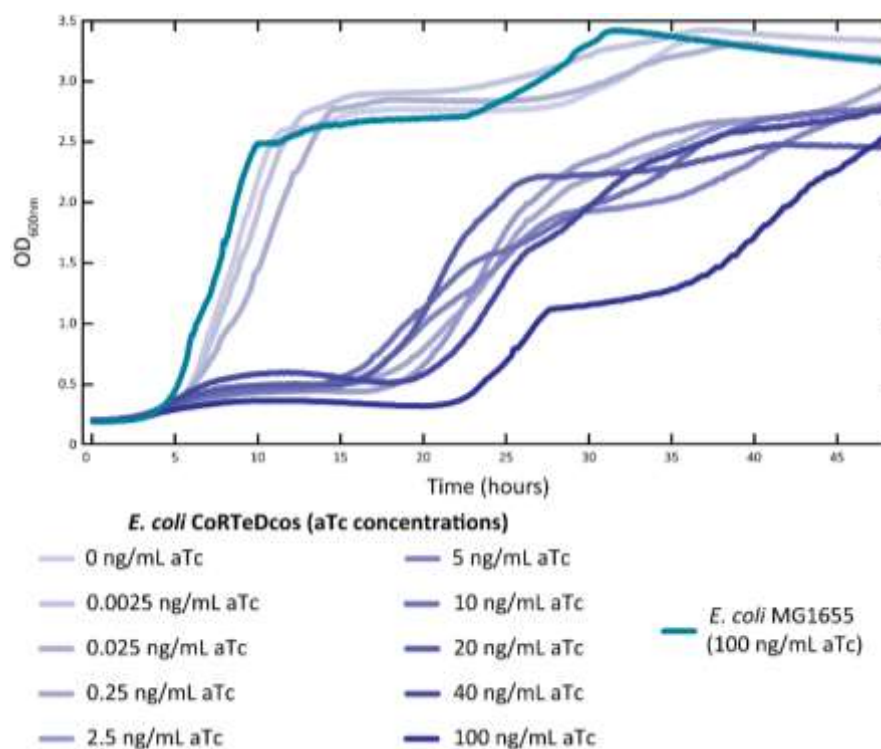


Figure S5.4. Growth curves of *E. coli* CoRteDcos in fast growth regime in different concentrations of aTc. The growth curve related to the wild-type is in presence of 100 ng/mL.

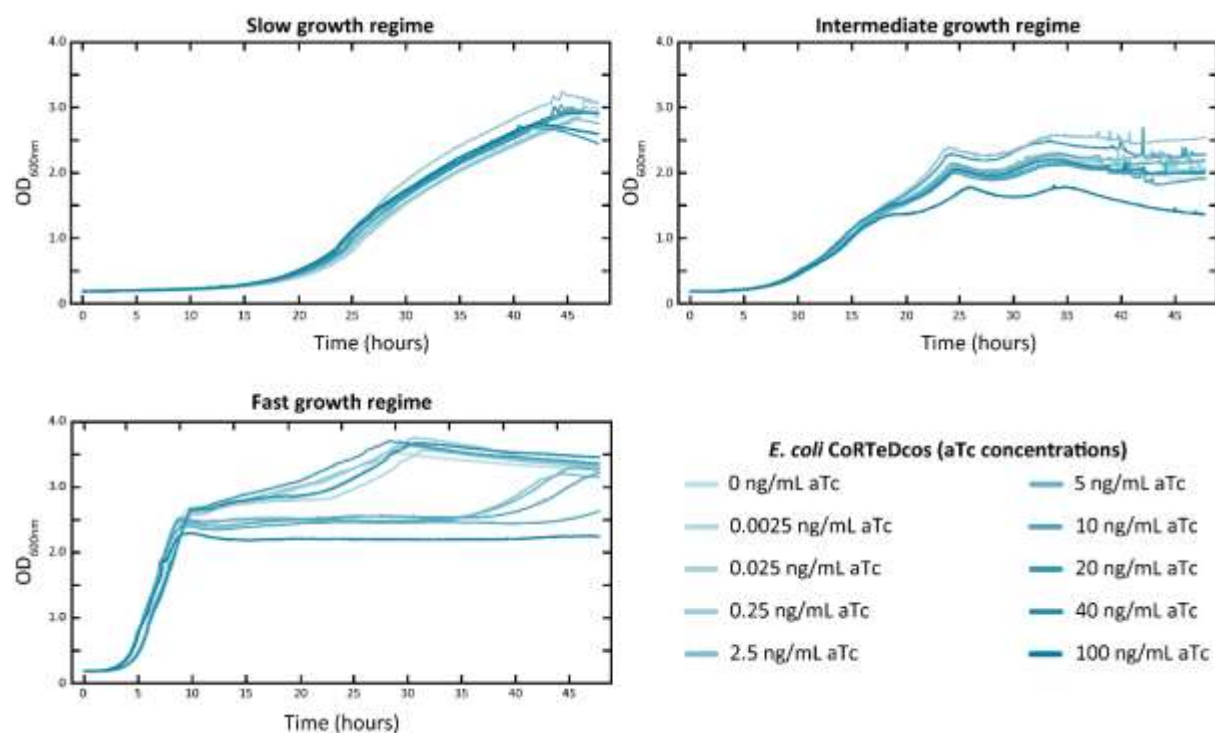


Figure S5.5. Growth curves of *E. coli* MG1655 in all growth regimes in different concentrations of aTc.

Table S5.4. List of mutations identified by whole genome sequencing in *E. coli* MG1655, *E. coli* CoRTeDcos and *E. coli* CoRTeDcos-R2G.

Strain	Location	Feature name	Feature function	Change	Variant frequency
<i>E. coli</i> M1655	276,825	<i>mmuP</i>	putative S-methylmethionine transporter	C -> A	100%
	4,298,541 - 4,298,542	Intergenic region	n.a.	+GC	99.10%
<i>E. coli</i> CoRTeDcos	276,825	<i>mmuP</i>	putative S-methylmethionine transporter	C -> A	100%
	4,298,541 - 4,298,542	Intergenic region	n.a.	+GC	99.10%
	3,885,155	Intergenic region <i>dnaA-dnaN</i>	n.a.	A -> C	100%
<i>E. coli</i> CoRTeDcos-R2G	276,825	<i>mmuP</i>	putative S-methylmethionine transporter	C -> A	100%
	4,298,541 - 4,298,542	Intragenic region	n.a.	+GC	99.10%
	3,885,155	Intergenic region <i>dnaA-dnaN</i>	n.a.	A -> C	100%
	3,929,441	<i>oriC</i>	Origin of replication	A -> G	100%

Source data

Source Data Table 5.1. Source data of the doubling times of *E. coli* MG1655 and *E. coli* CoRTeDcos in slow growth regime at different level of aTc induction, used for the bar plot in Figure 5.2A.

Strain	[aTc] (ng/mL)	Doubling time (min)	St. dev.
<i>E. coli</i> MG1655	100	168	6
	0	190.2	5.52
	0.0025	190.98	6.06
	0.025	178.98	6.06
	0.25	249	2.04
<i>E. coli</i> CoRTeDcos	2.5	259.8	3.66
	5	241.02	6.84
	10	235.02	3.24
	20	243	4.02
	40	229.98	2.94
	100	n.a.	n.a.

Source Data Table 5.2. Source data of the doubling times of *E. coli* MG1655 and *E. coli* CoRTeDcos in intermediate growth regime at different level of aTc induction, used for the bar plot in Figure 5.2B.

Strain	[aTc] (ng/mL)	Doubling time (min)	St. dev.
<i>E. coli</i> MG1655	100	79	1
	0	100.98	4.98
	0.0025	102	2.22
	0.025	105	2.52
	0.25	112.98	2.4
<i>E. coli</i> CoRTeDcos	2.5	132	1.56
	5	136.98	1.02
	10	130.98	1.02
	20	141	1.32
	40	142.98	1.86
	100	145.92	7.2

Source Data Table 5.3. Source data of the doubling times of *E. coli* MG1655 and *E. coli* CoRTeDcos in fast growth regime at different level of aTc induction, used for the bar plot in Figure 5.2C.

Strain	[aTc] (ng/mL)	Doubling time (min)	St. dev.
<i>E. coli</i> MG1655	100	42	0.6
	0	43.98	1.26
	0.0025	45	1.32
	0.025	46.98	2.4
	0.25	58.02	1.26
<i>E. coli</i> CoRTeDcos	2.5	58.02	4.32
	5	58.02	3.66
	10	55.98	1.26
	20	49.98	1.98
	40	52.02	1.98
	100	55.38	6.6

Source Data Table 5.4. Source data of number of origins of either the wild type *E. coli* MG1655 or the mutants *E. coli* CoRTeDcos and *E. coli* CoRTeDcos, in slow growth regime and at different concentrations of aTc, used for the bar plot in Figure 5.3C.

Strain	[aTc] (ng/mL)	Number of origins	St. dev.
<i>E. coli</i> MG1655	5	1.916004041	0.037837678
<i>E. coli</i> CoRTeDcos	0	1.899105546	0.045989166
	5	5.32941983	0.209887882
<i>E. coli</i> CoRTeDcos-R2G	0	1.853888065	0.050857973
	5	2.773263078	0.08756105

Source Data Table 5.5. Source data of asynchrony index of either the wild type *E. coli* MG1655 or the mutants *E. coli* CoRTeDcos and *E. coli* CoRTeDcos, in slow growth regime and at different concentrations of aTc, used for the bar plot in Figure 5.3C. The value used for *E. coli* MG1655 is taken from ³⁵⁷.

Strain	[aTc] (ng/mL)	Asynchrony index	St. dev.
<i>E. coli</i> MG1655	n.a.	0.1	0.04
<i>E. coli</i> CoRTeDcos	0	0.246851	0.008391617
	5	2.261816	0.094720993
<i>E. coli</i> CoRTeDcos-R2G	0	0.342532	0.074000626
	5	0.76162	0.074852273