# Supplemental material

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

| Strains | Characteristics | Source |
|---|---|---|
| *E. coli* MG1655 (wild type) | K-12; F⁻ λ⁻ *rph-1* | ATCC |
| *E. coli* DH5α | MG1655; *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF)U169 hsdR17* (r$_K^-$ m$_K^+$) | NEB |
| *E. coli* DH5α x pUC19_cat-pLtetO-1-TetR | DH5α harbouring the pUC19_cat-pLtetO-1-TetR plasmid. | This study |
| *E. coli* DH5α x pUC19_dnaAcos-KanR | DH5α harbouring the pUC19_dnaAcos-KanR plasmid | This study |
| *E. coli* DH5α x pSC020 | DH5α harbouring the pSC020 plasmid | This study |
| *E. coli* MG1655 x pSC020 | MG1655 harbouring the pSC020 plasmid | This study |
| *E. coli* pre-CoRTeDcos x pSC020 | MG1655 harbouring the pSC020 plasmid; Δ*DnaAp2*, Δ*dnaA::lox66-cmR-lox71-pLtetO-1-tetR-dnaAcos-lox66-kanR-lox71* | This study |
| *E. coli* CoRTeDcos x pSC020 | MG1655 harbouring the pSC020 plasmid; Δ*DnaAp2*, Δ*dnaA::lox-pLtetO-1-tetR-dnaAcos-lox* | This study |
| *E. coli* CoRTeDcos | MG1655; Δ*DnaAp2*, Δ*dnaA::lox-pLtetO-1-tetR-dnaAcos-lox* | This study |
| *E. coli* CoRTeDcos-R2G | MG1655; Δ*DnaAp2*, Δ*dnaA:: pLtetO-1-tetR-dnaAcos;* A-to-G mutation in box R2 of *oriC* | This study |

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

| Plasmid | Characteristics | Source |
|---|---|---|
| pSC020 | *repA101, oriV, ampR, araC, P$_{araB}$-Red, lacI$^q$, P$_{Lac}$-Cre* | [353] |
| pUC19_cat-pLtetO-1-TetR | pUC19, *ampR, lox66-cmR-lox71, pLtetO-1-tetR* | This study |
| pUC19_dnaAcos-kanR | pUC19, *ampR, dnaAcos, lox66-kanR-lox71* | This study |

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

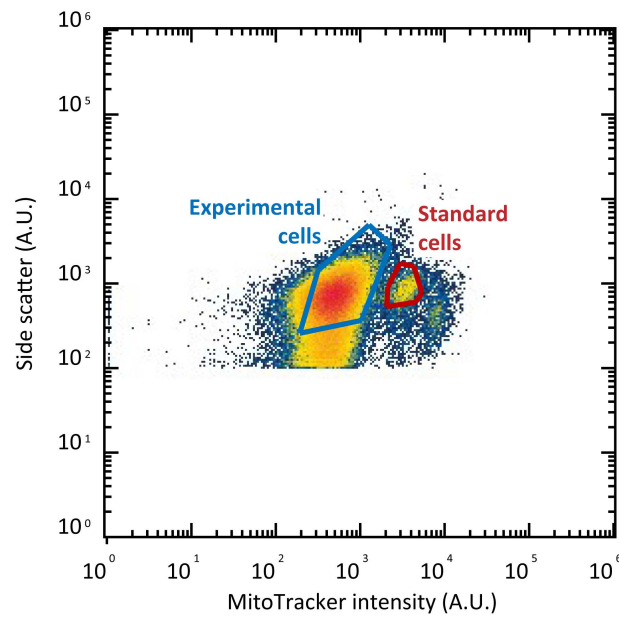| Identifier | Sequence (5'-3') | Used for |
|---|---|---|
| BG22166 | cgagtggagtccgcc**a**tgtcactttcgctttggcagcagtgtcttgcccgattgcagg atgagttaccagccacagaattcagtatgtggatacgcccattgcaggcggaactg agcgataacacgctggccctgtacgcgccaaaccgttttgtcctcgattgggtacgg gacaagtaccttaataatatcaatggactgctaaccagtttctgcggagcggatgcc ccacagctgcgttttgaagtcggcaccaaaccggtgacgcaaacgccacaagcgg cagtgacgagcaacgtcgcggccctgcacaggtggcgcaaacgcagccgcaac gtgctgcgccttctacgcgctcaggttgggataacgtcccggccccggcagaaccg acctatcgttctaacgtaaacgtcaaacacacgtttgataacttcgttgaaggtaaat ctaaccaactggcgcgcgcggcggctcgccTTgtggcggataaccctggcggtgc ctataacccgttgttcctttatggcggcacgggtctgggtaaaactcacctgctgcat gTTgtgggtaacggcattatggcgcgcaagccgaatgccaaagtggtttatatgc actccgagcgctttgttcaggacatggttaaagccctgcaaaacaacgcgatcgaa gagtttaaacgctactaccgttccgtagatgcactgctgatcgacgatattcagttttt tgctaataaagaacgatctcaggaagagtttttcTATaccttcaacgccctgctgga aggtaatcaacagatcattctcacctcggatcgcCACccgaaagagatcaacggc gttgaggatcgtttgaaatcccgcttcggttgggggactgactgtggcgatcgaaccg ccagagctggaaacccgtgtggcgatcctgatgaaaaaggccgacgaaaacga cattcgtttgccgggcgaagtggcgttctttatcgccaagcgtctacgatctaacgta cgtgagctggaaggggcgctgaaccgcgtcattgccaatgccaactttaccggac gggcgatcaccatcgacttcgtgcgtgaggcgctgcgcgacttgctggcattgcag gaaaaactggtcaccatcgacaatattcagaagacggtggcggagtactacaag atcaaagtcgcggatctcctttccaagcgtcgatcccgctcggtggcgcgtccgcgc cagatggcgatggcgctggcgaaagagctgactaaccacagtctgccggagatt ggcgatgcgtttggtggccgtgaccacacgacggtgcttcatgcctgccgtaagatc gagcagttgcgtgaagagagccacgatatcaaagaagattttttcaaatttaatcag aacattgtcatcgtaaacctat | Synthetic DNA of *dnaAcos* |
| BG22660 | **gcctcgcggcaggatcgtttacacttagcgagttctggaaagtcctgtgg**gctagtt attgctcagcggtgg | Introduced upstream homology arm to the *lox66-cat-lox71-pLtetO-1-tetR-BsaI* fragment. |
| BG20764 | gcgtttcggtgatgacggtgaaaac | Amplified the *lox66-cat-lox71-pLtetO-1-tetR-BsaI* fragment, together with BG22660. |
| BG21406 | cgattcattaatgcagctggcacg | Amplified the *BsaI-dnaAcos-lox66-kanR-lox71* fragment, together with BG22661. |
| BG22661 | **taaatgctcacgttctacggtaaatttcataggtttacgatgacaatgtt**ccgactgg aaagctaccg | Introduced downstream homology arm to *dnaAcos-lox66-kanR-lox71* fragment. |
| BG22467 | cggcaatttccgcgccttc | Used for colony PCR and Sanger sequencing of the *dnaA* locus. |
| BG22496 | gacctcacaccagtggaaaccag | Used for colony PCR and Sanger sequencing of the *dnaA* 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.
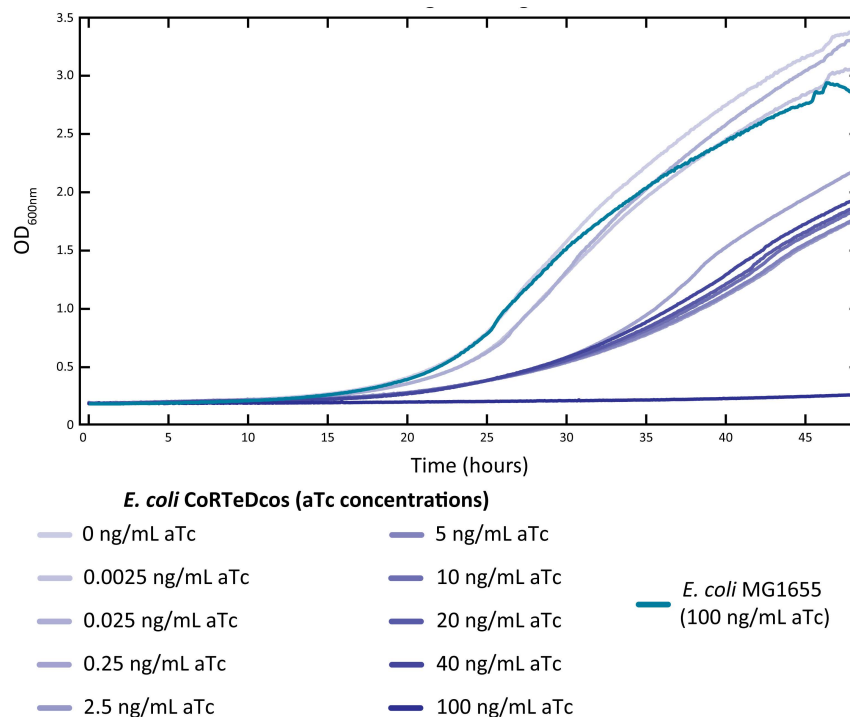


*E. coli* **CoRTeDcos (aTc concentrations)**

| | |
|---|---|
| 0 ng/mL aTc | 5 ng/mL aTc |
| 0.0025 ng/mL aTc | 10 ng/mL aTc |
| 0.025 ng/mL aTc | 20 ng/mL aTc |
| 0.25 ng/mL aTc | 40 ng/mL aTc |
| 2.5 ng/mL aTc | 100 ng/mL aTc |

*E. coli* MG1655 (100 ng/mL aTc)

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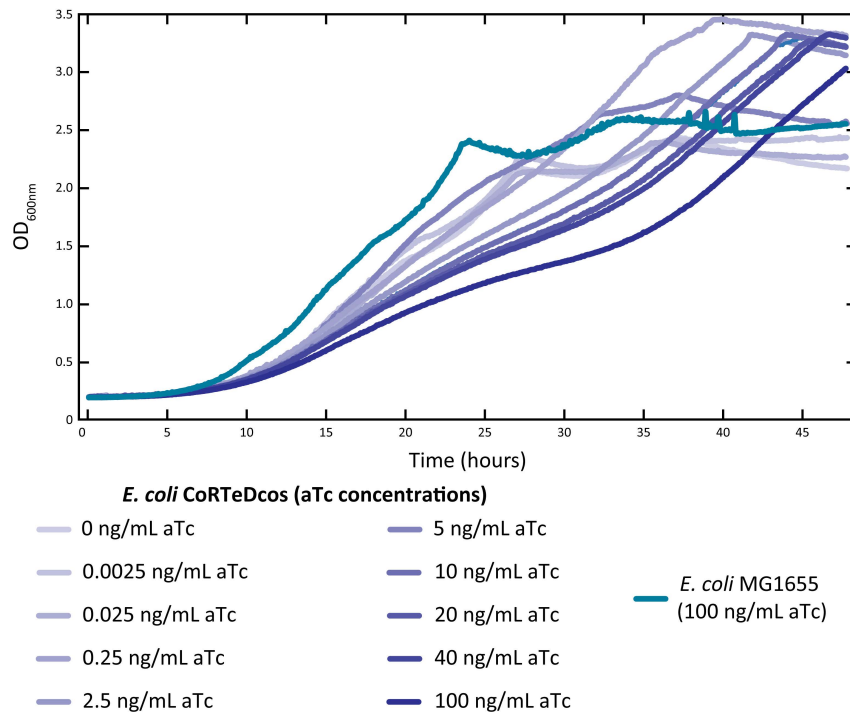
**Figure S5.3. Growth curves of _E. coli CoRTeDcos_ in intermediate growth regime in different concentrations of aTc**. The growth curve related to the wild-type is in presence of 100 ng/mL.
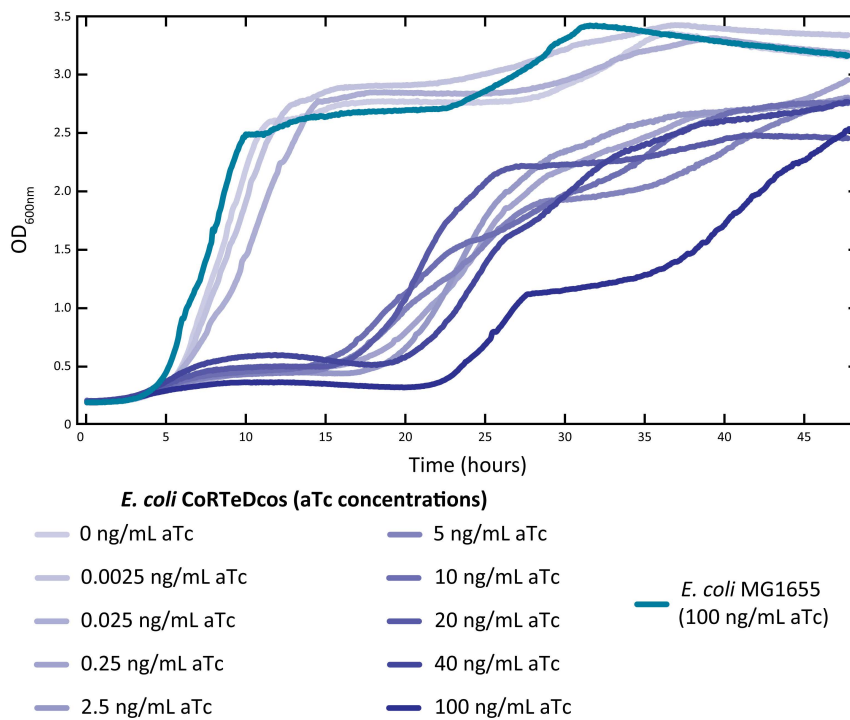


**Figure S5.4. Growth curves of _E. coli_ CoRTeDcos in fast growth regime in different concentrations of aTc.** The growth curve related to the wild-type is in presence of 100 ng/mL.
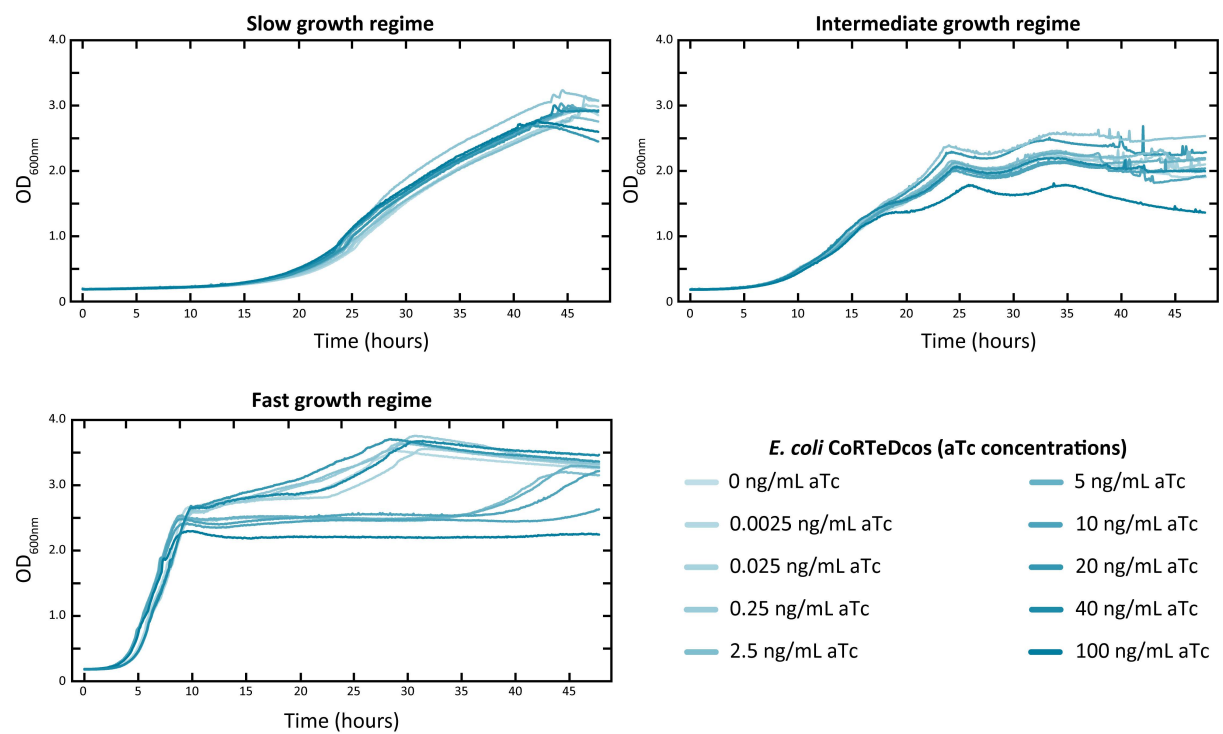
**Figure S5.5.** Growth curves of *E. coli* MG1655 in all growth regimes in different concentrations of aTc.

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 |
|---|---|---|---|---|---|
| *E. coli* M1655 | 276,825 | *mmuP* | putative S-methylmethionine transporter | C -> A | 100% |
| | 4,298,541 - 4,298,542 | Intergenic region | n.a. | +GC | 99.10% |
| *E. coli* CoRTeDcos | 276,825 | *mmuP* | 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 *dnaA-dnaN* | n.a. | A -> C | 100% |
| *E. coli* CoRTeDcos-R2G | 276,825 | *mmuP* | 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 *dnaA-dnaN* | n.a. | A -> C | 100% |
| | 3,929,441 | *oriC* | 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. |
|---|---|---|---|
| *E. coli* MG1655 | 100 | 168 | 6 |
| *E. coli* CoRTeDcos | 0 | 190.2 | 5.52 |
| | 0.0025 | 190.98 | 6.06 |
| | 0.025 | 178.98 | 6.06 |
| | 0.25 | 249 | 2.04 |
| | 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. |
|---|---|---|---|
| *E. coli* MG1655 | 100 | 79 | 1 |
| *E. coli* CoRTeDcos | 0 | 100.98 | 4.98 |
| | 0.0025 | 102 | 2.22 |
| | 0.025 | 105 | 2.52 |
| | 0.25 | 112.98 | 2.4 |
| | 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. |
|---|---|---|---|
| *E. coli* MG1655 | 100 | 42 | 0.6 |
| *E. coli* CoRTeDcos | 0 | 43.98 | 1.26 |
| | 0.0025 | 45 | 1.32 |
| | 0.025 | 46.98 | 2.4 |
| | 0.25 | 58.02 | 1.26 |
| | 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. |
|---|---|---|---|
| *E. coli* MG1655 | 5 | 1.916004041 | 0.037837678 |
| *E. coli* CoRTeDcos | 0 | 1.899105546 | 0.045989166 |
| | 5 | 5.32941983 | 0.209887882 |
| *E. coli* 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. |
|---|---|---|---|
| *E. coli* MG1655 | n.a. | 0.1 | 0.04 |
| *E. coli* CoRTeDcos | 0 | 0.246851 | 0.008391617 |
| | 5 | 2.261816 | 0.094720993 |
| *E. coli* CoRTeDcos-R2G | 0 | 0.342532 | 0.074000626 |
| | 5 | 0.76162 | 0.074852273 |