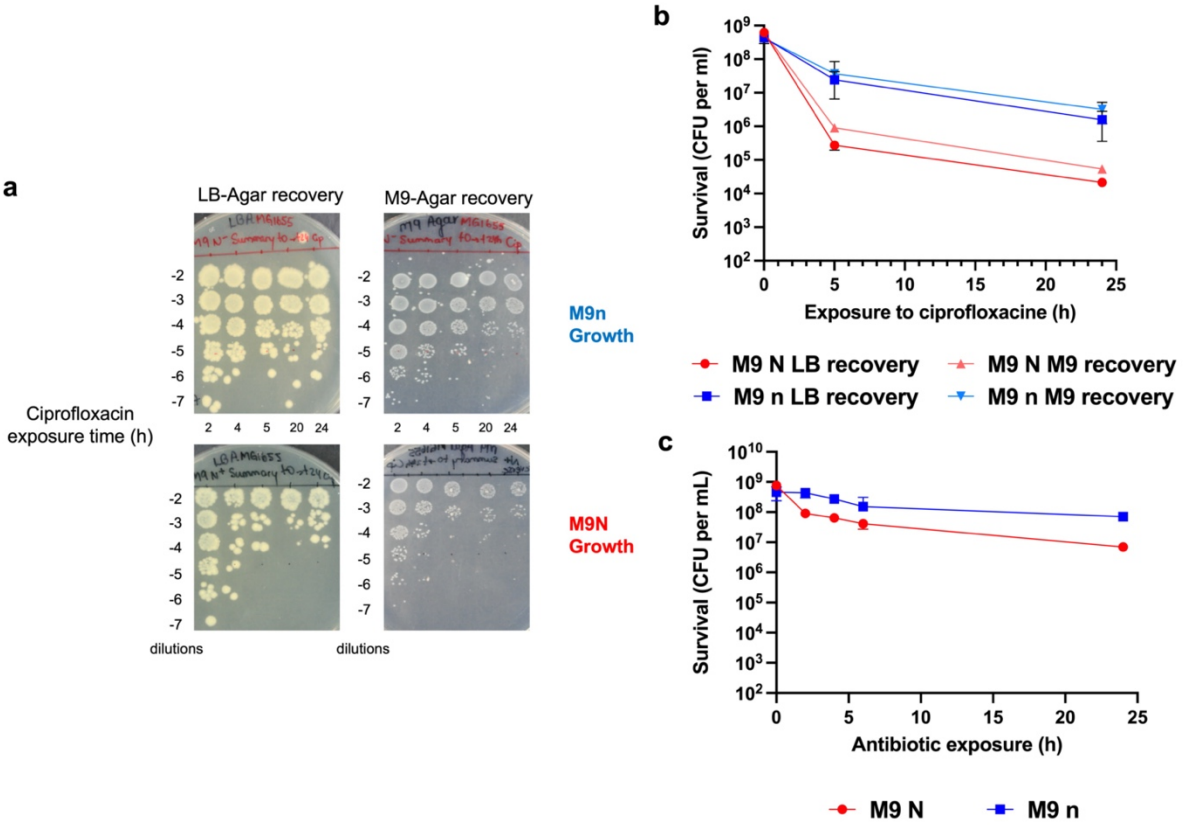
Extended Data and Tables

This file contains:

- Extended Data Figures 1 to 4
- Extended Tables 1 to 4
- Captions for Extended Tables 1 to 4
- References for supplementary materials

Extended Data figures

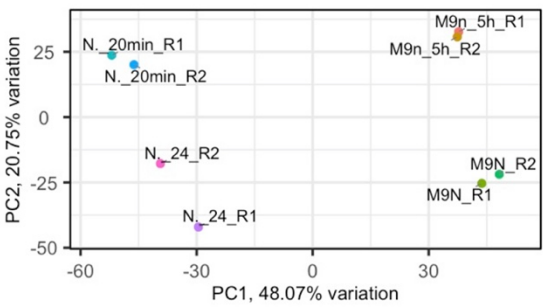


Extended Data Fig. 1: Recovery in rich or poor media does not significantly impact CFU counts of *E. coli*. (**a**) CFU plates of ciprofloxacin-exposed *E. coli* cultured 3 h in M9N (blue) or M9n (red) before plating on M9 agar or LB agar plates. 3 h after media switch to M9 N or M9 n, *E. coli* cultures were treated with 10x MIC of ciprofloxacin. Bacterial killing was monitored by plating for CFU on LB agar or M9 agar. 5 µL drops of dilutions from 10^{-2} to 10^{-5} were dispensed onto M9 agar or LB agar after 2, 4, 5, 20 and 24 h of exposure to 10x MIC of ciprofloxacin. (**b**) Time-kill curve of ciprofloxacin treated *E. coli* grown in M9 N or M9 n (SD = 3). 3 h after media switch into M9 N (red) or M9 n (blue), *E. coli* cultures were treated with ciprofloxacin at 10x MIC. Bacterial killing over time was tracked by CFU counting after recovery on LB (dark red and blue) or M9 (light red and blue) agar plates. (**c**) Time-kill curve of stationary phase *E. coli* (M9 N or M9 n) treated with ciprofloxacin at 10x MIC (SD =3). *E. coli* cultures at $OD600_{nm}$ = 0.2 were grown 18 h in M9 N (red) or M9 n (blue) before treatment with ciprofloxacin. Survival was measured over time by CFU counts on LB agar plates.

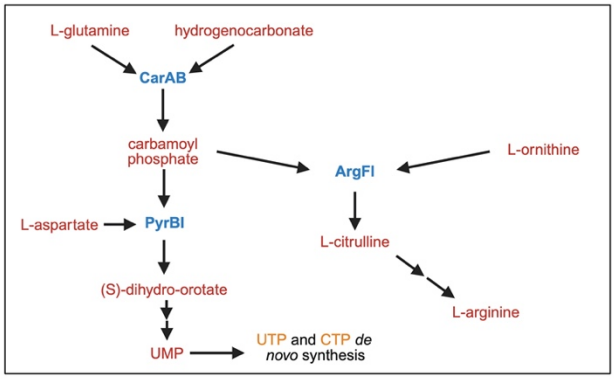
a

M9n 5h			N starvation 20 min			N starvation 24h		
Gene	Log_2FC	pValue	Gene	Log_2FC	pValue	Gene	Log_2FC	pValue
pyrB	-10.17	1.3E-05	***gatB***	-8.62	9.7E-33	***metE***	-6.48	3.0E-10
flgB	-10.01	6.6E-08	***pyrB***	-8.59	2.1E-43	***pyrB***	-6.23	7.1E-07
pyrI	-7.62	8.2E-08	***carA***	-8.18	7.9E-35	***pyrI***	-6.14	7.8E-08
carA	-6.80	1.8E-07	***purE***	-8.12	6.1E-29	***gatB***	-5.93	5.0E-05
borD	-6.62	1.4E-07	***gatA***	-8.11	8.7E-47	***gatA***	-5.77	4.0E-07
metE	-6.54	7.0E-06	***metE***	-7.88	3.0E-35	***carA***	-5.72	1.6E-10
carB	-6.39	1.5E-06	***borD***	-7.85	5.1E-41	***proV***	-5.65	4.1E-10
gatB	-6.35	9.6E-08	***purK***	-7.38	2.2E-27	***carB***	-5.58	5.2E-14
ilvX	-6.13	6.9E-05	***rplC***	-7.16	7.3E-20	***ilvC***	-5.31	4.1E-15
yoeI	-5.90	8.5E-04	***rpsJ***	-7.06	2.5E-18	***borD***	-5.08	6.5E-04
fruB	-5.74	3.9E-07	***carB***	-6.90	1.1E-25	***pyrL***	-5.07	3.4E-04
mdtJ	-5.38	6.6E-06	***rplD***	-6.83	1.4E-17	***proW***	-5.06	4.3E-13
cheA	-5.37	1.6E-06	***pyrI***	-6.76	4.5E-29	***aldA***	-4.99	4.2E-10
malK	-5.16	4.2E-07	***yfdI***	-6.66	7.0E-25	***artJ***	-4.87	4.7E-10
ompF	-5.07	2.0E-05	***rplA***	-6.62	4.0E-17	***ndk***	-4.75	1.3E-09
yidD	-5.02	9.4E-06	***rplB***	-6.51	3.2E-14	***rpsI***	-4.74	5.1E-07
mgtA	-4.93	2.5E-04	***rplW***	-6.51	2.0E-22	***malK***	-4.68	6.0E-07
argI	-4.89	3.2E-07	***purC***	-6.39	2.6E-16	***nmpC***	-4.60	7.8E-08
malE	-4.85	2.4E-07	***ilvC***	-6.34	8.0E-23	***proX***	-4.55	3.1E-09
rplM	-4.84	5.9E-06	***pyrL***	-6.29	5.7E-18	***rplM***	-4.51	9.7E-09

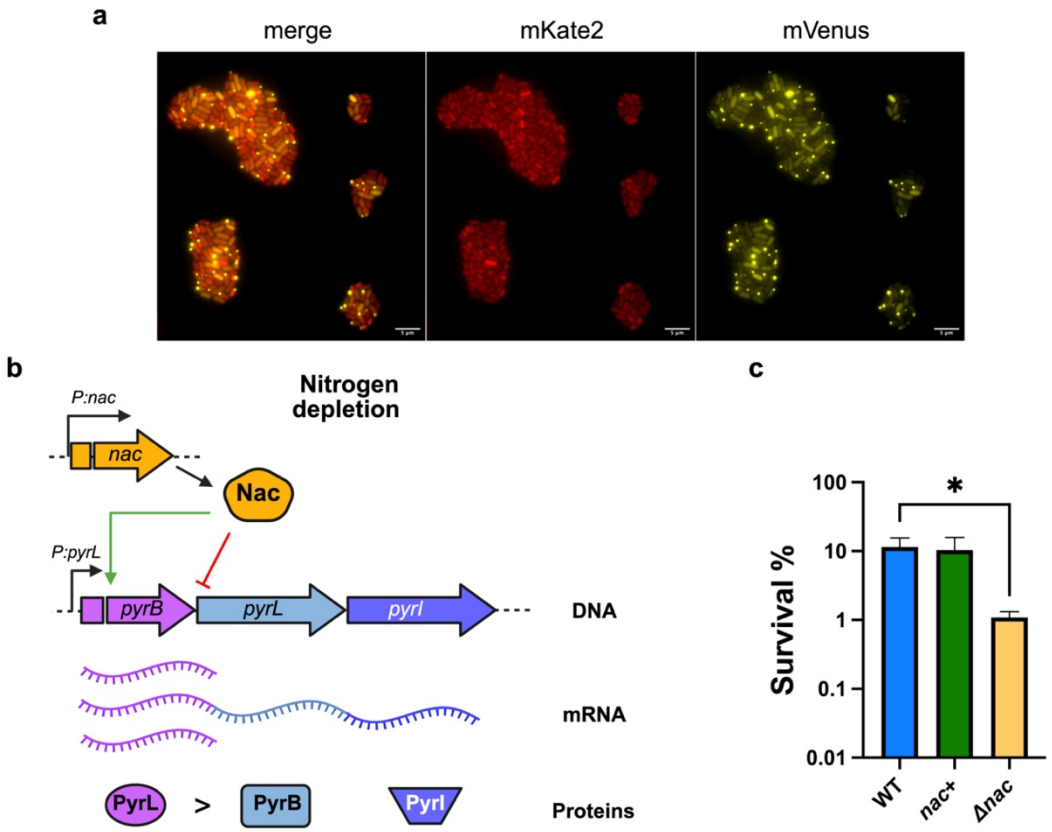
b



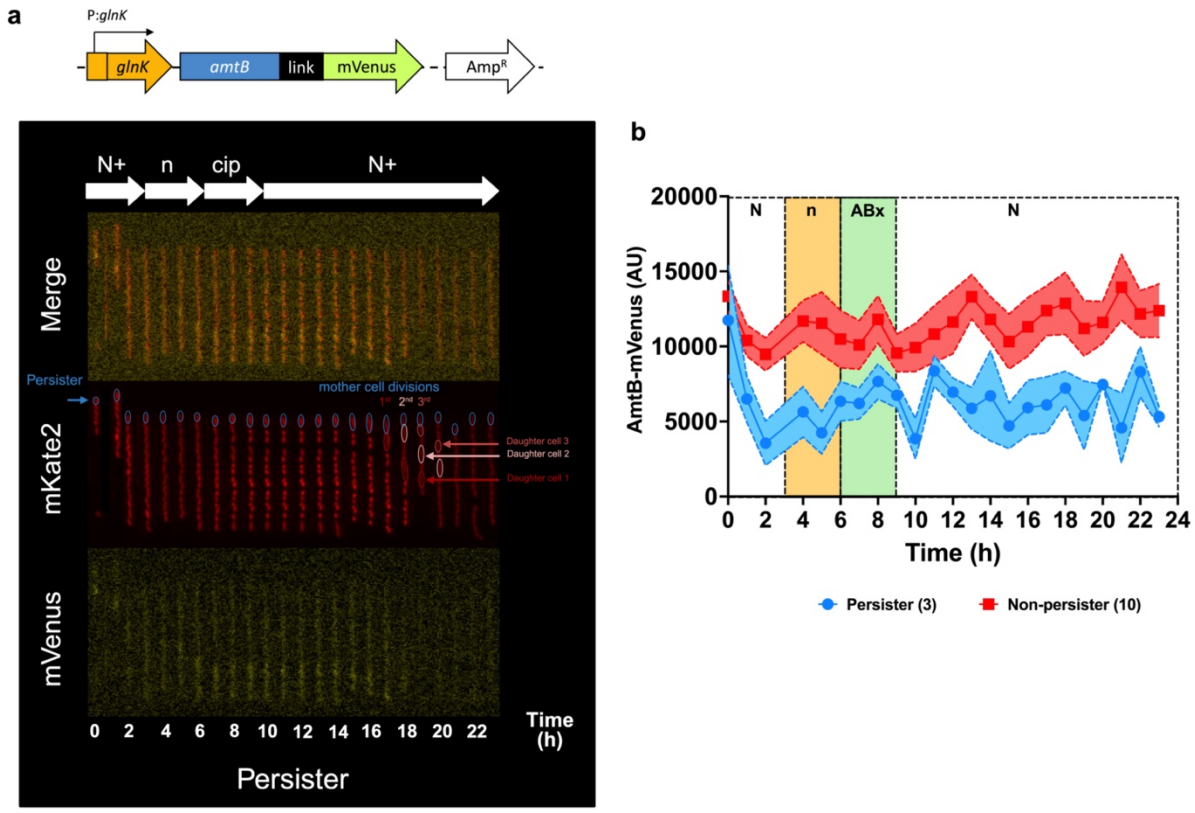
c



Extended Data Fig. 2: *E. coli* transcriptomic response to nitrogen starvation.
(**a**) Bottom 20 list of downregulated genes in response to multiple nitrogen starvation. Genes differentially expressed from nitrogen rich versus nitrogen starved (5 h, 20 min or 24 h) conditions were compared. Genes were ordered from the lowest fold change and *pyrB*, *pyrI*, *carA* and *carB* are highlighted in bold red. (**b**) Principal Component Analysis of datasets obtained for multiple nitrogen depletion transcriptomes. Total gene counts from RNA-seq data from exponential phase (M9N) and 5h nitrogen starved (M9n 5h) cultures were compared to Switzer *et al.* 2018 datasets (N- 20 min and N- 24 h). Each dot represents one replicate. (**c**) PyrBI and CarAB functions in the superpathway of histidine, purine and pyrimidine biosynthesis. CarAB and PyrBI are both involved in the uridine-5'-monophosphate (UMP) synthesis pathway which converts L-glutamine, L-aspartate and bicarbonate into UMP. Pyrimidine synthesis is also interconnected to L-arginine synthesis pathway through carbamoyl phosphate as a shared precursor[1]. UMP can be converted directly into Uridine-5'-triphosphate (for RNA synthesis) or Cytosine-5'-triphosphate (for DNA and RNA synthesis). Proteins are annotated in bold blue, metabolites in burgundy and nucleotides in yellow.



Extended Data Fig. 3: Nac overexpression in response to nitrogen depletion is responsible for *pyrBI* downregulation in persisters. (**a**) Micrograph of *E. coli* DE32 transformed with a *pyrBI*-mVenus translational reporter. *pyrBI* transcriptional unit was cloned in frame with mVenus into the backbone of a low copy plasmid pMMB67EH. The pMMB-*pyrBI*-mVenus reporter was transformed into the DE32 strain harboring mKate fluorophore in the genome. Cells were observed by fluorescent microscopy on a M9 N agarose pad at 100x magnification. (**b**) Regulation of *pyrLBI* transcription by Nac. (Top) Transcriptional regulation of *pyrLBI* by Nac. In response to nitrogen starvation, expression of Nac is enhanced by NtrBC activation. This dual transcriptional regulator binds to two positions on *pyrLBI* operon, enhancing *pyrL* and repressing *pyrBI* transcription, causing a disbalance in protein production[2,3]. (**c**) *nac* deletion decreases persister formation under nitrogen starvation. *E. coli* BW25113*Δnac* is from the KEIO collection (yellow). *nac* overexpression results from induction by Atet (200 nM) during media switch (green). Ciprofloxacin was then added at 10X MIC, and after 5 hours, surviving persisters were determined by cfu count. The bargraph show the survival compared to BW25113 strain bearing no plasmid (blue) to ciprofloxacin exposure for 5 h at 37°C. Values are means ± SDs (n=3).



Extended Data Fig. 4: AmtB expression is modestly reduced in persisters. (**a**) (top) The natural operon comprised of *glnK*, *amtB* and the associated promoter were fused to mVenus allowing to track expression. The resulting transcriptional unit was inserted in pMMB-67EH. (bottom) Kymographs of an *E. coli* pMMB-amtB-mVenus persister after ciprofloxacin exposure. The mKate2 signal allowed to determine a mask to identify cells. Persisters are circled in blue. Red, beige and coral circles highlight daughter cells produced from the persister mother cell after removal of the antibiotic. (**b**) Quantification of AmtB-mVenus in persisters (blue) and non-persisters (red). Area around the curve represents standard deviation. 26250 cells were screened. mVenus fluorescence of mother cells was quantified every hour by fluorescence microscopy at a magnification of 40x.

Extended Data and Tables

Extended table 1: Strains and plasmids
Extended table 2: List of primers
Extended table 3: List of commonly downregulated genes in response to nitrogen starvation (intersection of our and Switzer *et al*, data)
Extended table 4: List of commonly upregulated genes in response to nitrogen starvation (intersection of our and Switzer *et al*, data)

References for Extended Materials

1 Hove-Jensen, B. *et al.* Phosphoribosyl Diphosphate (PRPP): Biosynthesis, Enzymology, Utilization, and Metabolic Significance. *Microbiol Mol Biol Rev* **81** (2017).

2 Park, J. Y. *et al.* Model-driven experimental design workflow expands understanding of regulatory role of Nac in Escherichia coli. *NAR Genom Bioinform* **5**, lqad006 (2023).

3 Baumgart, L. A. *et al.* Persistence and plasticity in bacterial gene regulation. *Nat Methods* **18**, 1499-1505 (2021).