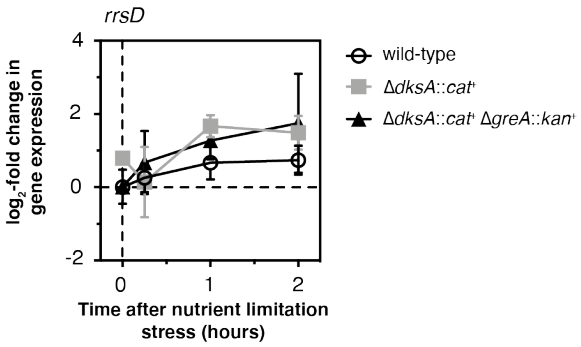

FIG S1 16S rRNA (*rrsD*) expression increases under the nutrient limitation stress conditions used in this paper. *E. coli* MG1655 wild-type and isogenic Δ*dksA1000*::*cat*$^+$, and Δ*dksA1000*::*cat*$^+$ Δ*greA788*::*kan*$^+$ strains were grown at 37°C to A_{600}=0.2–0.4 in rich medium (LB) and then shifted to minimal medium (MOPS with no amino acids, 4 g l^{-1} glucose, 0.1 mM K_2HPO_4, 0.1 mM uracil) for 2 hours. qRT-PCR was used to measure fold changes in transcript abundance, relative to the wild-type at t = 0 h, at the indicated timepoints (n=3, ± SD).

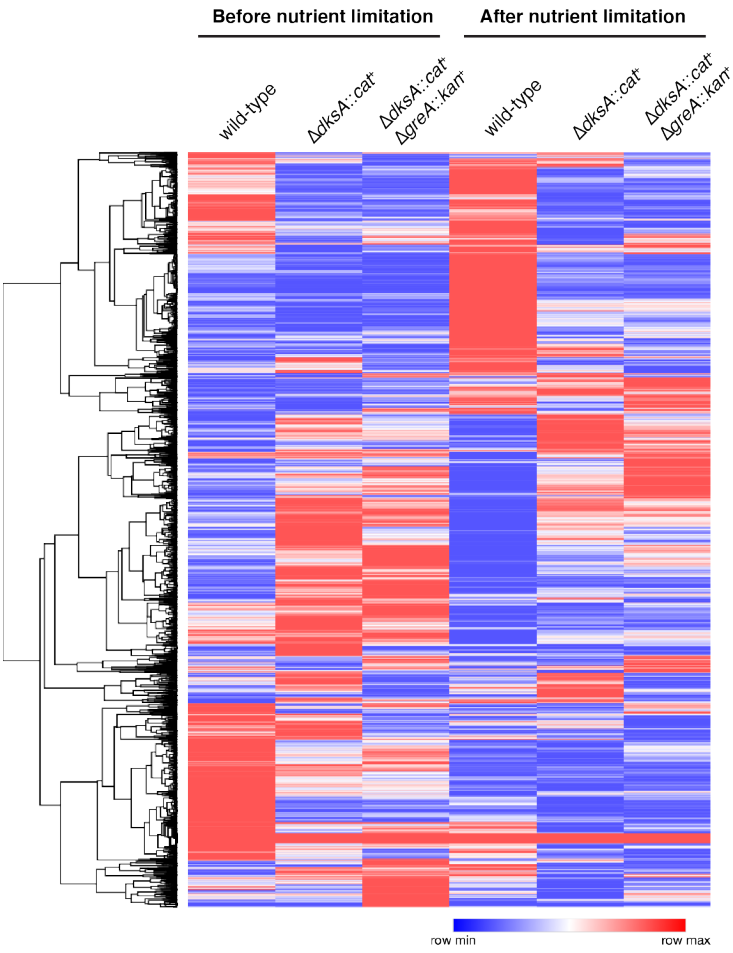

FIG S2 Heatmap of RNA sequencing data. *E. coli* strains MG1655, MJG1419 (Δ*dksA1000*::*cat*$^+$), and MJG1561 (Δ*dksA1000::cat*$^+$ Δ*greA788::cat*$^+$) were grown at 37°C to A_{600}=0.2–0.4 in rich medium (LB)(black circles) and then shifted to minimal medium (MOPS with no amino acids, 4 g l^{-1} glucose, 0.1 mM K_2HPO_4) for 5 minutes (n = 1). Heatmap was generated by hierarchical clustering of normalized gene expression values (from Supplemental Dataset S1) with Morpheus (https://software.broadinstitute.org/morpheus), using the default parameters.

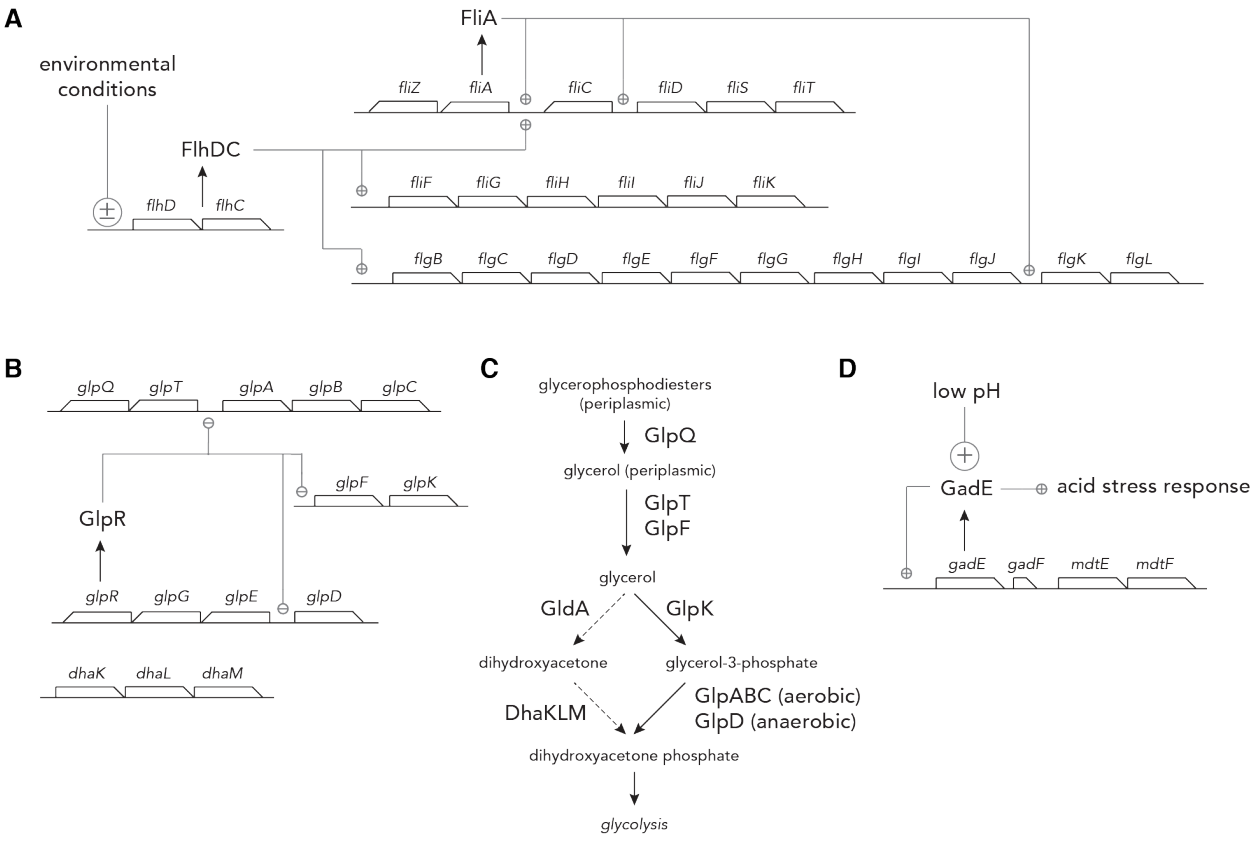

FIG S3 Select *E. coli* operons and pathways regulated by nutrient limitation, *dksA*, and *greA*. (1) (*A*) Operons for flagellar regulation and biosynthesis. (*B*) Operons for glycerol dissimilation and regulation thereof. (*C*) The *E. coli* pathway for glycerol uptake and dissimilation. Dashed lines indicate an alternative pathway active in the absence of GlpK activity. (*D*) The *gadE* operon, which includes the multidrug efflux pump-encoding *mdtEF* genes.

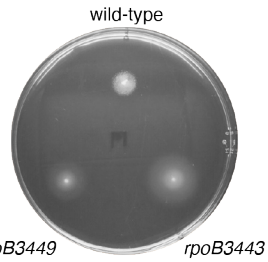

FIG S4 Stringent mutations of RNA polymerase lead to enhanced motility. *E. coli* MG1655 wild-type and isogenic *rpoB3449* and *rpoB3443* strains were inoculated into LB containing 0.25% agar and incubated at 37°C (representative of 3 independent experiments).

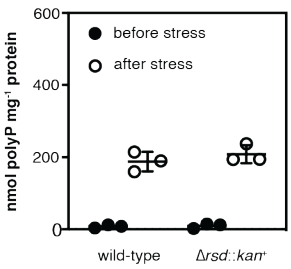

FIG S5 An *rsd* null mutation does not affect polyP synthesis. *E. coli* MG1655 wild-type and isogenic Δ*rsd-766*::*kan*$^+$ strains were grown at 37°C to A_{600}=0.2–0.4 in rich medium (LB)(black circles) and then shifted to minimal medium (MOPS with no amino acids, 4 g l^{-1} glucose, 0.1 mM K_2HPO_4) for 2 hours (white circles)(n=3, ± SD). PolyP concentrations are in terms of individual phosphate monomers. No significant difference was found between polyP levels in the mutant and wild-type strains (two-way repeated measures ANOVA with Holm-Sidak's multiple comparisons test).

DATASET S1 Results of RNA sequencing of wild-type, *dksA*, and *dksA greA* mutants before and after nutrient limitation stress. *E. coli* strains MG1655, MJG1419 (Δ*dksA1000*::*cat*$^+$), and MJG1561 (Δ*dksA1000::cat*$^+$ Δ*greA788::ca*t$^+$) were grown at 37°C to A_{600}=0.2–0.4 in rich medium (LB)(black circles) and then shifted to minimal medium (MOPS with no amino acids, 4 g l^{-1} glucose, 0.1 mM K_2HPO_4) for 5 minutes (n = 1). Gene expression values (RPKM normalized to the upper quartile of gene expression) were determined using Rockhopper 2 (2), with the default parameters.

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1. Keseler IM, Mackie A, Santos-Zavaleta A, Billington R, Bonavides-Martinez C, Caspi R, Fulcher C, Gama-Castro S, Kothari A, Krummenacker M, Latendresse M, Muniz-Rascado L, Ong Q, Paley S, Peralta-Gil M, Subhraveti P, Velazquez-Ramirez DA, Weaver D, Collado-Vides J, Paulsen I, Karp PD. 2017. The EcoCyc database: reflecting new knowledge about Escherichia coli K-12. Nucleic Acids Res 45:D543-D550.
2. Tjaden B. 2015. De novo assembly of bacterial transcriptomes from RNA-seq data. Genome Biol 16:1.