

Supplementary materials for:

**The complex logic of stringent response regulation in
Caulobacter crescentus: starvation signaling in an oligotrophic
environment**

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Table S1. List of genes regulated in carbon starvation microarray.... TableS1.xls

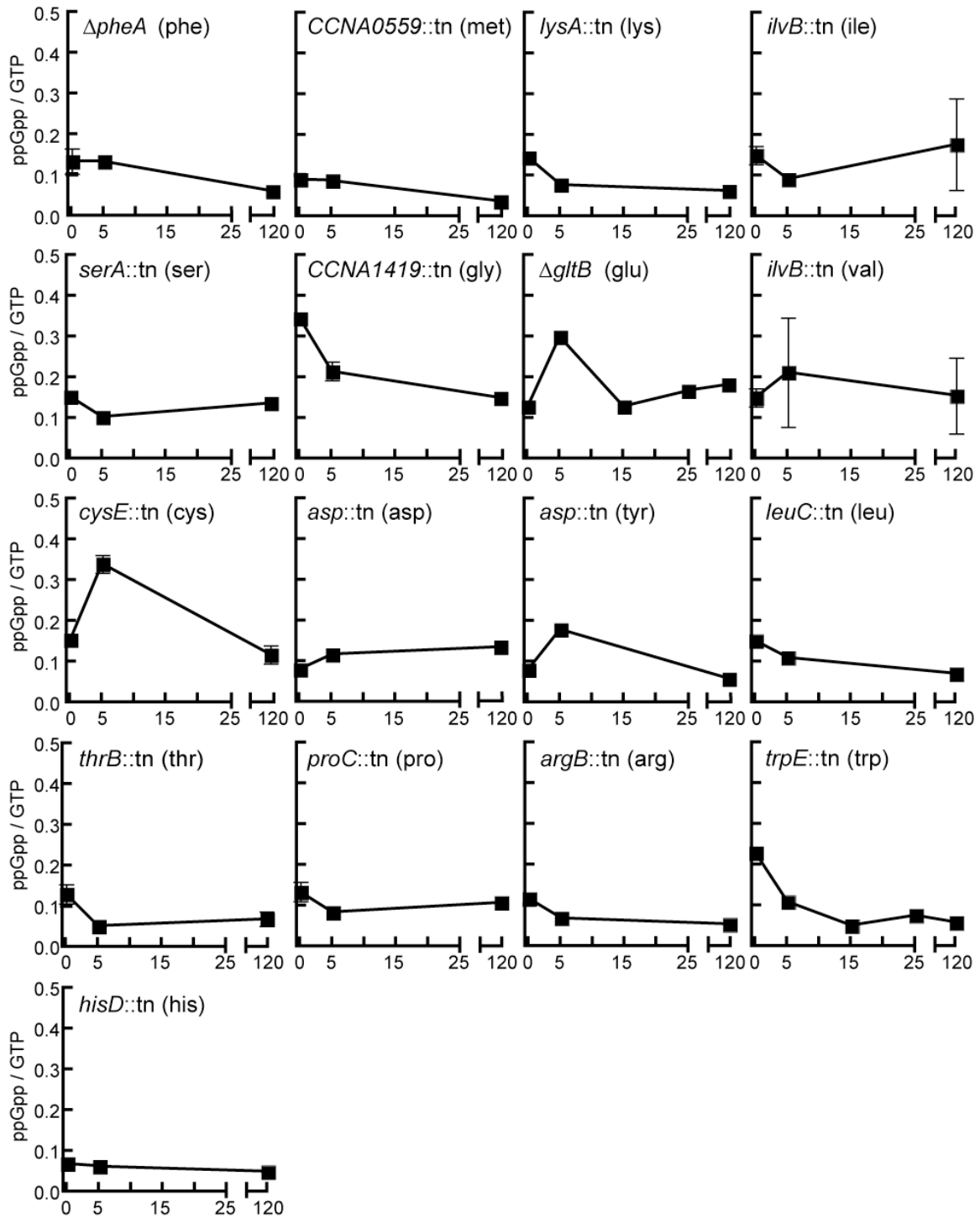
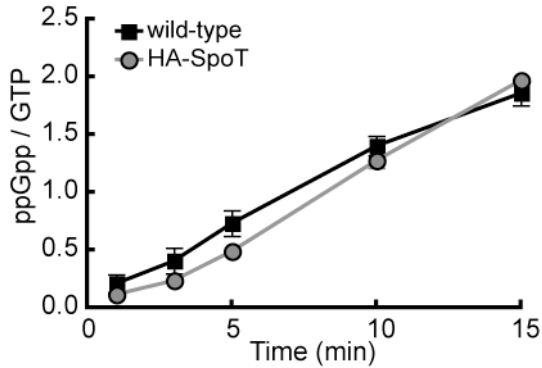


Figure S1. ppGpp accumulation upon amino-acid-starvation of auxotrophs. Each panel shows the ppGpp/ GTP ratio before (0 min) and at 5 and 120 minutes after amino acid starvation. In each experiment the strain is auxotrophic for the amino acid indicated in parentheses, either because of a transposon insertion disruption or an in-frame deletion of an amino acid biosynthetic gene. Each auxotroph was grown in M2G with the indicated amino acid at 100 $\mu\text{g/ml}$ and alanine at 200 $\mu\text{g/ml}$ (to relieve growth inhibition). Cells were then labeled in

$\text{KH}_2^{32}\text{PO}_4$ and the indicated amino acid was washed out of the medium to induce starvation. Note that the scale differs from that in Figure 1B.

A. ppGpp in glucose starvation



B. Sucrose cushion fractionation of cytoplasmic protein LovK-HA

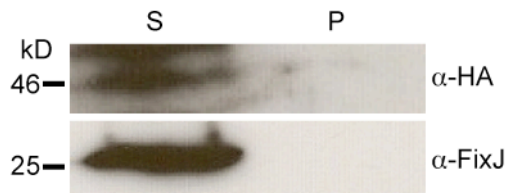
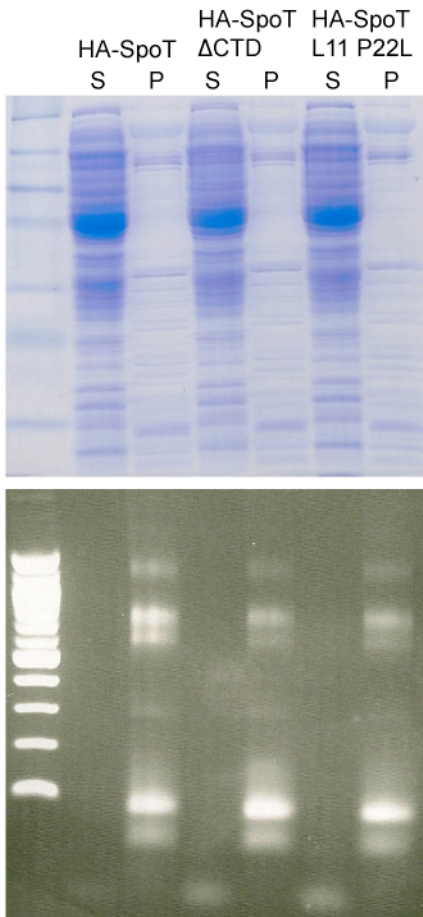


Figure. S2. HA controls. A) The HA-SpoT strain can accumulate ppGpp upon carbon starvation like the wild-type. N=2, error bars refer to standard deviation. B) Western blots of supernatant (S) and pellet (P) fractions from a cell lysate of a strain with LovK-HA spun through a 1M sucrose cushion to separate the ribosomes from other cell constituents. The HA tag does not confer ribosome-copurification upon the cytoplasmic histidine kinase LovK. FixJ is used as a non-ribosome associated control.

A. Protein and RNA in sucrose cushion assays



B. HA-SpoT stability

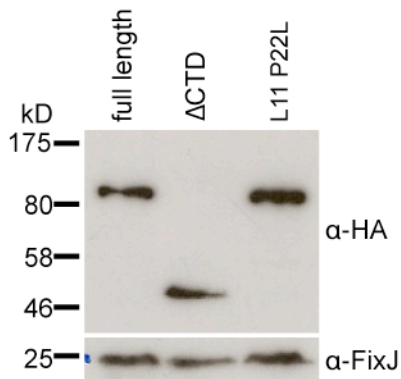


Figure. S3. Sucrose cushion assay controls for Fig. 2B. A) Top: coomassie-stained polyacrylamide gel of soluble and pellet fractions; bottom: ethidium bromide-stained agarose gel of phenol / chloroform extractions of soluble and pellet fractions. B) Western blot of cell culture aliquots before sucrose cushion assay was conducted showing similar levels of HA-SpoT in the strains. FixJ is used as a loading control.

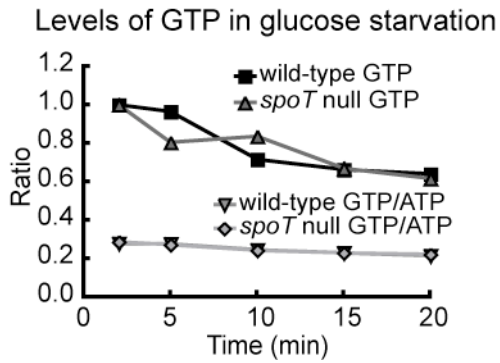


Figure S4. Relative GTP levels in starved cells. GTP levels during glucose starvation in wild-type and a SpoT H67A suppressor which has a transposon disrupting the *spoT* locus. The cells were grown in M2G, labeled in M2GL + $\text{KH}_2^{32}\text{PO}_4$ for two hours, then washed and resuspended in M2L + $\text{KH}_2^{32}\text{PO}_4$ to induce glucose starvation. An equal volume of culture was taken at each time point for TLC analysis. The top data traces are the counts from the GTP spots divided by the count at the first time point ($t=2$) for each strain. The bottom data traces are the counts from the GTP spots divided by the counts from the ATP spots for each sample.