The CBS subdomain of inosine 5'-monophosphate dehydrogenase regulates purine nucleotide turnover

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SUPPLEMENTARY MATERIAL

Figure S1. A ClustalW alignment of *E. coli* BW25113 *guaB*$^{+}$, *E. coli* MP101 *guaB*$^{\Delta CBS}$ and *Streptococcus pyogenes* IMPDH proteins. Amino acid identities are outlined. The deleted CBS domain sequence is marked with arrows. The “scar” sequence replacing the CBS domain sequence in MP101 is underlined.

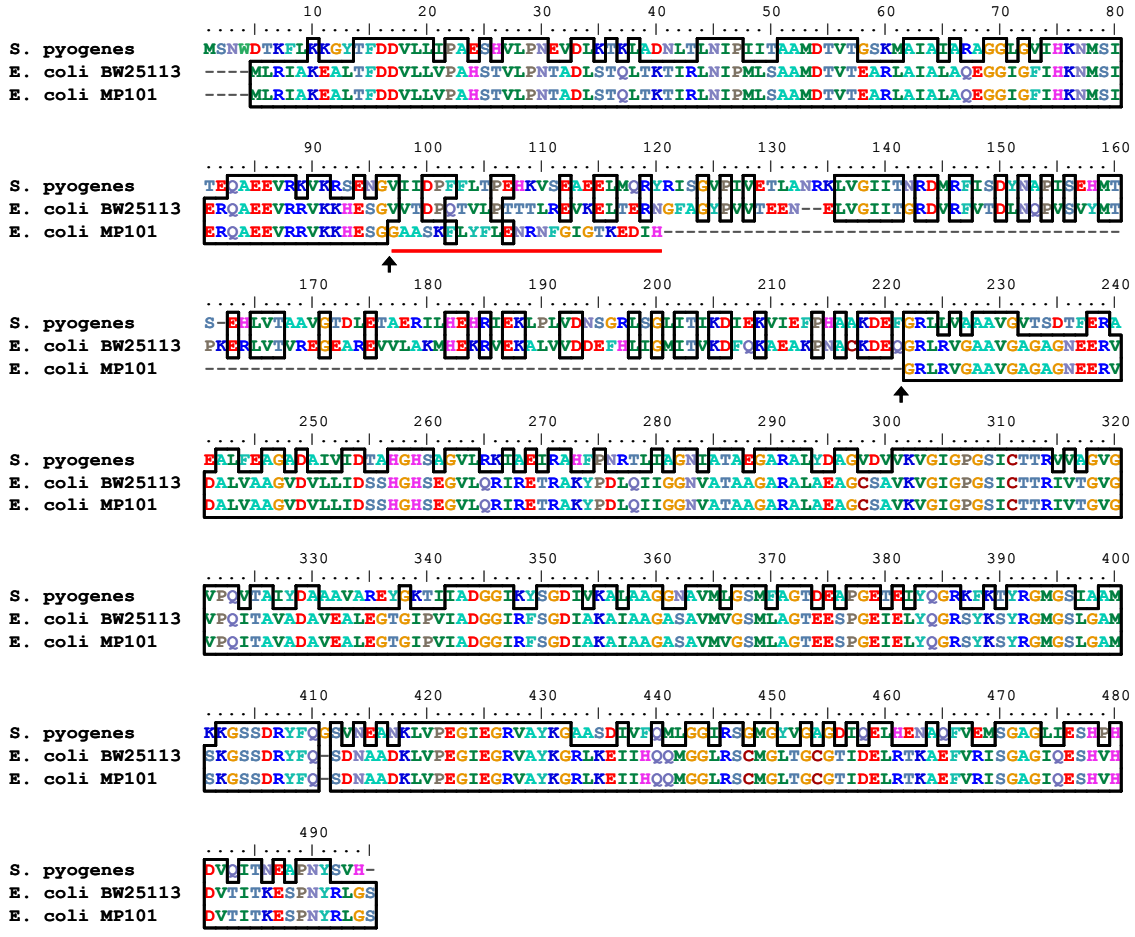

Figure S2. Cloning of the *guaB*$^{\Delta CBS}$ gene in pCR2.1-TOPO vector under the control of a constitutive lacUV5 promoter and native 5´-UTR. The 5´ part of the sequence cloned is presented along with the 5´ flanking pCR2.1-TOPO sequence. The forward PCR primer used for gene amplification before TOPO cloning is underlined. The lacUV5 promoter sequence is highlighted.

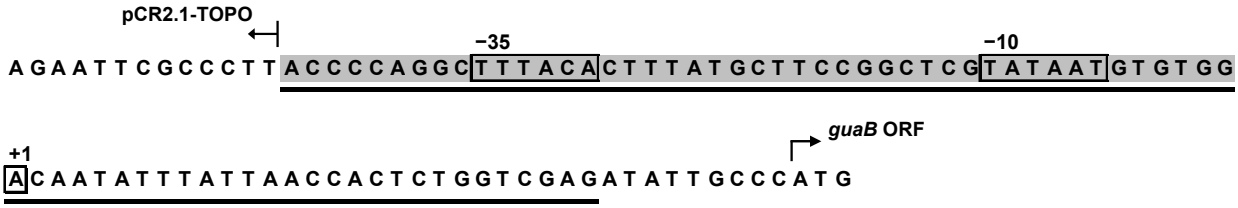

Figure S3. Growth rates of BW25113 and MP101 with and without guanine supplementation. BW25113 *guaB*$^{+}$ and MP101 *guaB*$^{\Delta CBS}$ were grown in liquid MOPS culture. Each culture was continuously pumped through a closed circuit system consisting of a 50-ml culture flask and a 1-ml spectrophotometer flow cell. An OD_{600} reading was taken every 2 min. 60 µg/ml guanosine was added at the time indicated by the arrow. Guanosine was used in place of guanine due to better solubility.

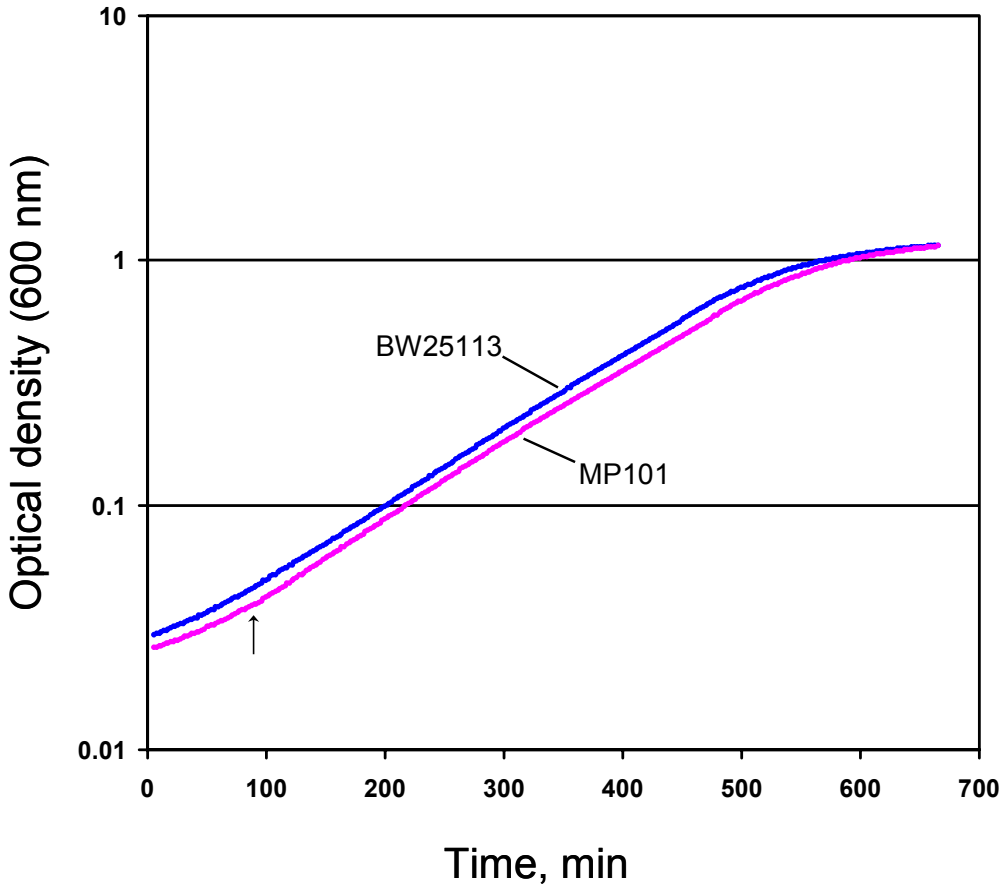

Figure S4. Responses of the BW25113 *guaB*$^{+}$ and MP101 *guaB*$^{\Delta CBS}$ nucleotide pools to purine bases, nucleosides and inhibitors. Blue diamonds, ATP; red circles, GTP, green squares, ATP/GTP ratio. The concentrations used were as follows: adenine, 30 µg/ml; hypoxanthine, 30 µg/ml; guanosine, 60 µg/ml; chloramphenicol, 50 µg/ml; decoyinine, 100 µg/ml. Vertical axis, nucleotide pool (mM); horizontal axis, time from base/nucleoside/inhibitor addition (min).

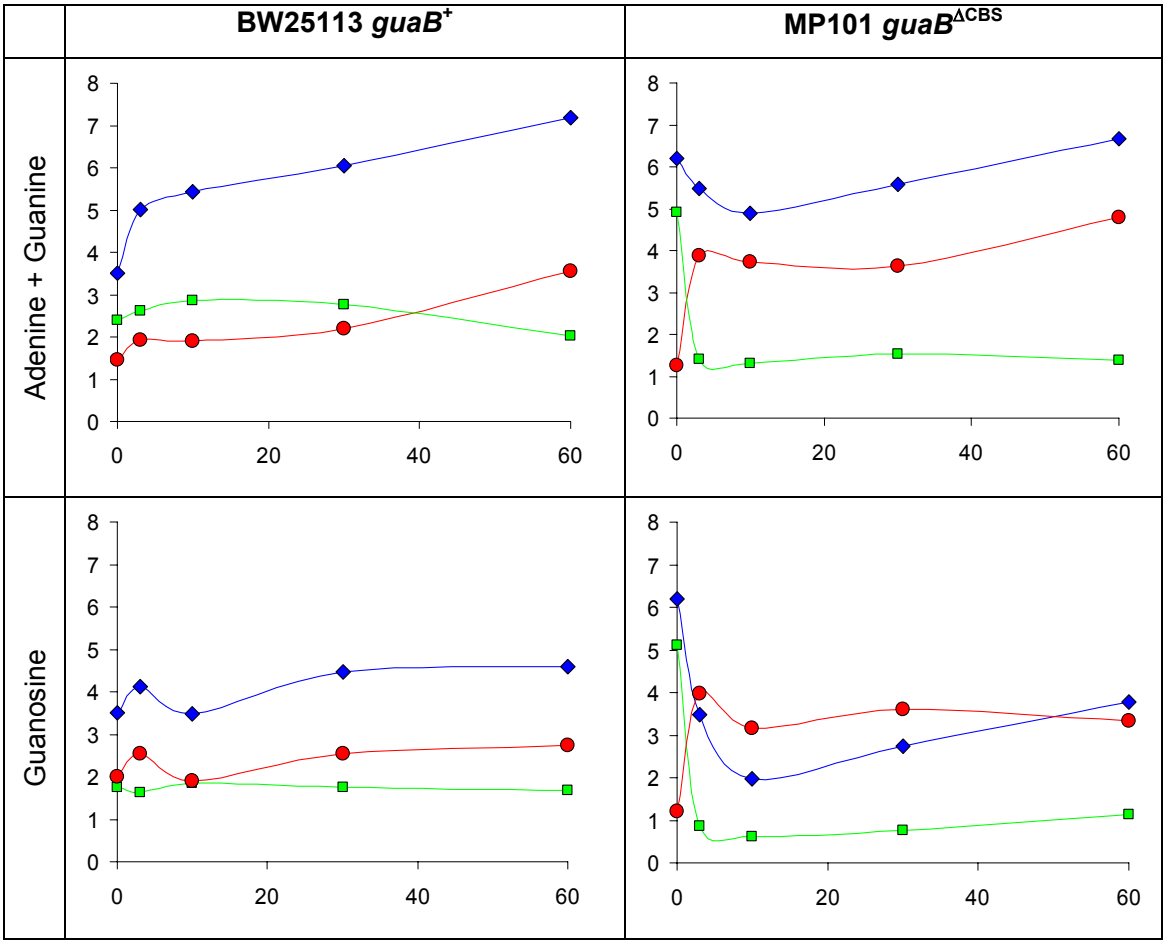

Figure S4, *contd*.

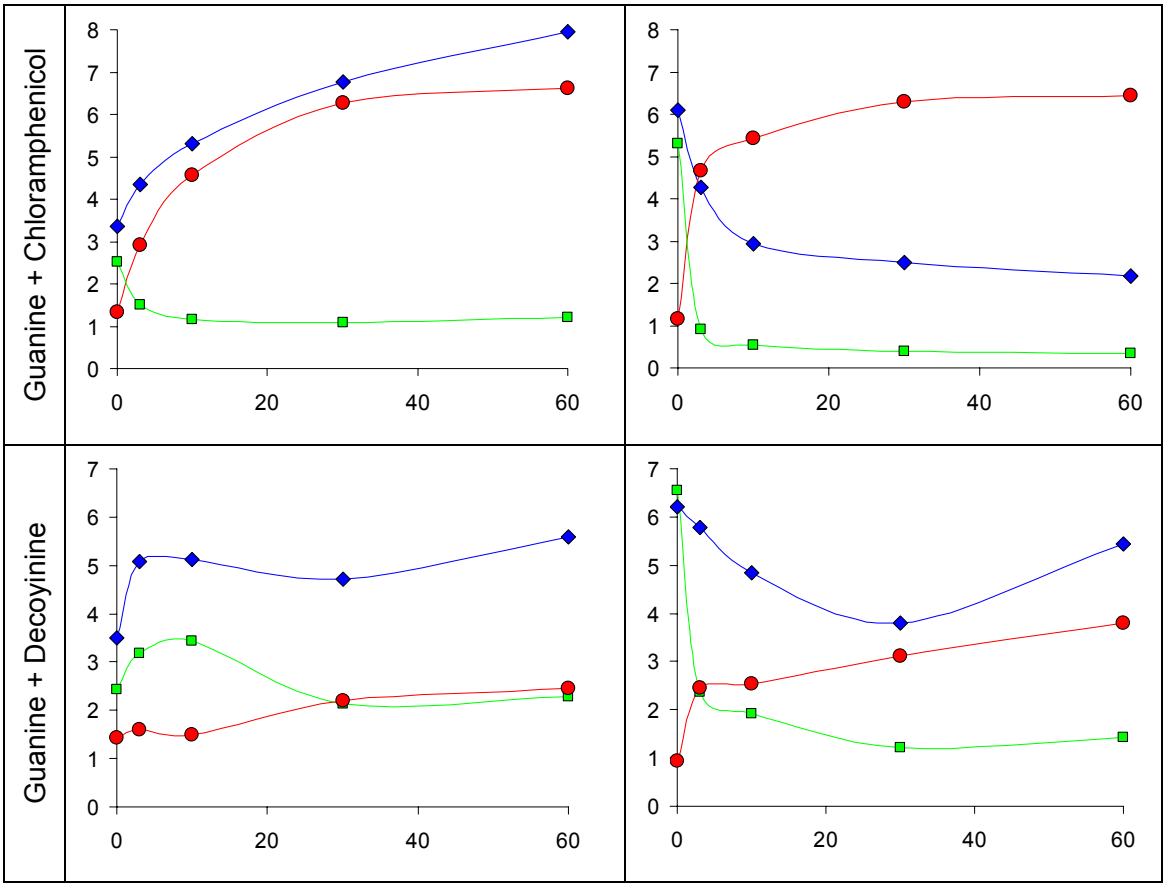

Figure S5. Complementation of the mutant nucleotide pool phenotype by *guaB*$^{+}$ gene present *in trans*. *A*. Nucleotide pools of MP101/pGUAB (*guaB*$^{\Delta CBS}$/ *guaB*$^{+}$) and MP101/pGUA4 (*guaB*$^{\Delta CBS}$/ *guaB*$^{\Delta CBS}$) during steady-state growth on MOPS minimal media. ***B***. Changes in nucleotide pools induced by addition of 30 µg/ml guanine at 0 min.

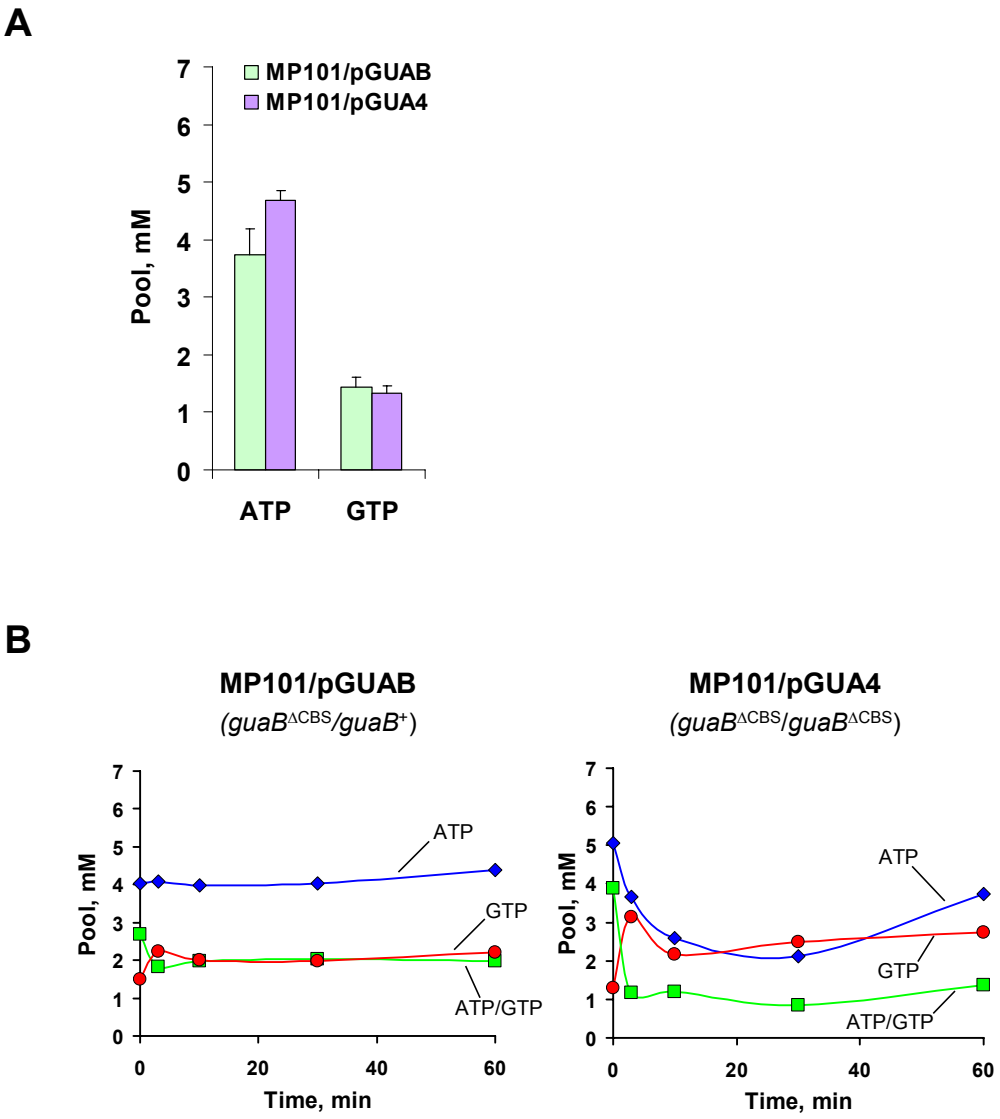

Figure S6. GMP synthetase activity in crude extracts of BW25113 *guaB*$^{+}$ and MP101 *guaB*$^{\Delta CBS}$ grown on minimal media and supplemented with 10 µg/ml xanthine. Cells were grown to a mid-exponential phase in minimal MOPS media or MOPS media supplemented with 10 µg/ml xanthine and harvested by centrifugation. BugBuster reagent (Novagen) containing 30 µg/ml PMSF was used for protein extraction (1 ml per 100 OU_{600}). The extract was cleared by centrifugation and GMPS activity per mg of protein was measured as described in the *Experimental Procedures* section. GMPS activity is expressed as percent of the activity observed in the BW25113 strain grown on minimal media. An average of 4 measurements is given. Error bars are standard deviations.