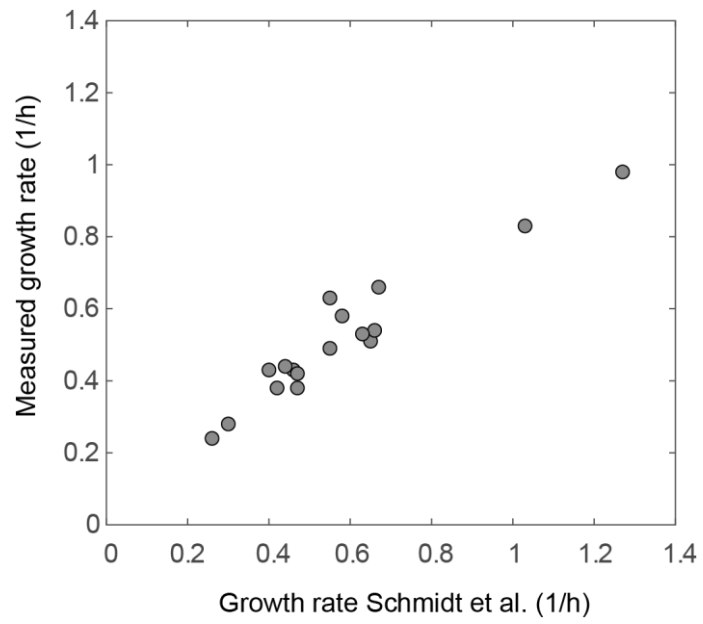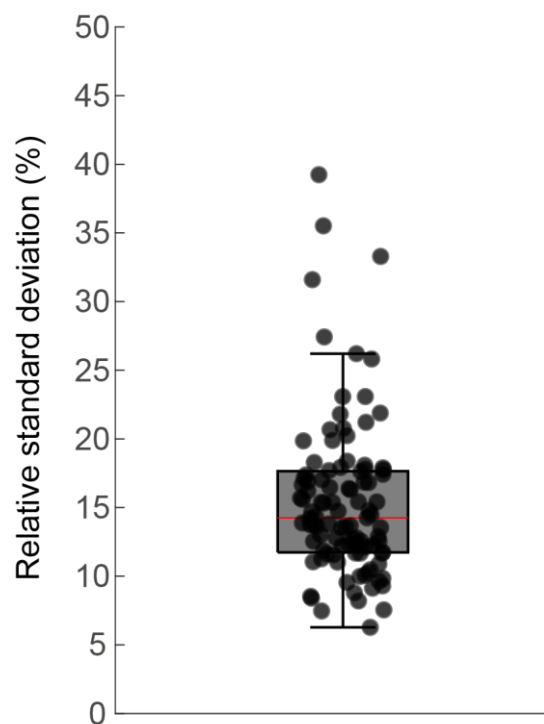## Supplemental information

Homeostasis of the biosynthetic *E. coli* metabolome

Dušica Radoš, Stefano Donati, Martin Lempp, Johanna Rapp, and Hannes Link

Figure S1. Growth rates in 19 conditions measured in this study and growth rates reported in the proteome study (Schmidt et al., 2016). Related to Figure 1.
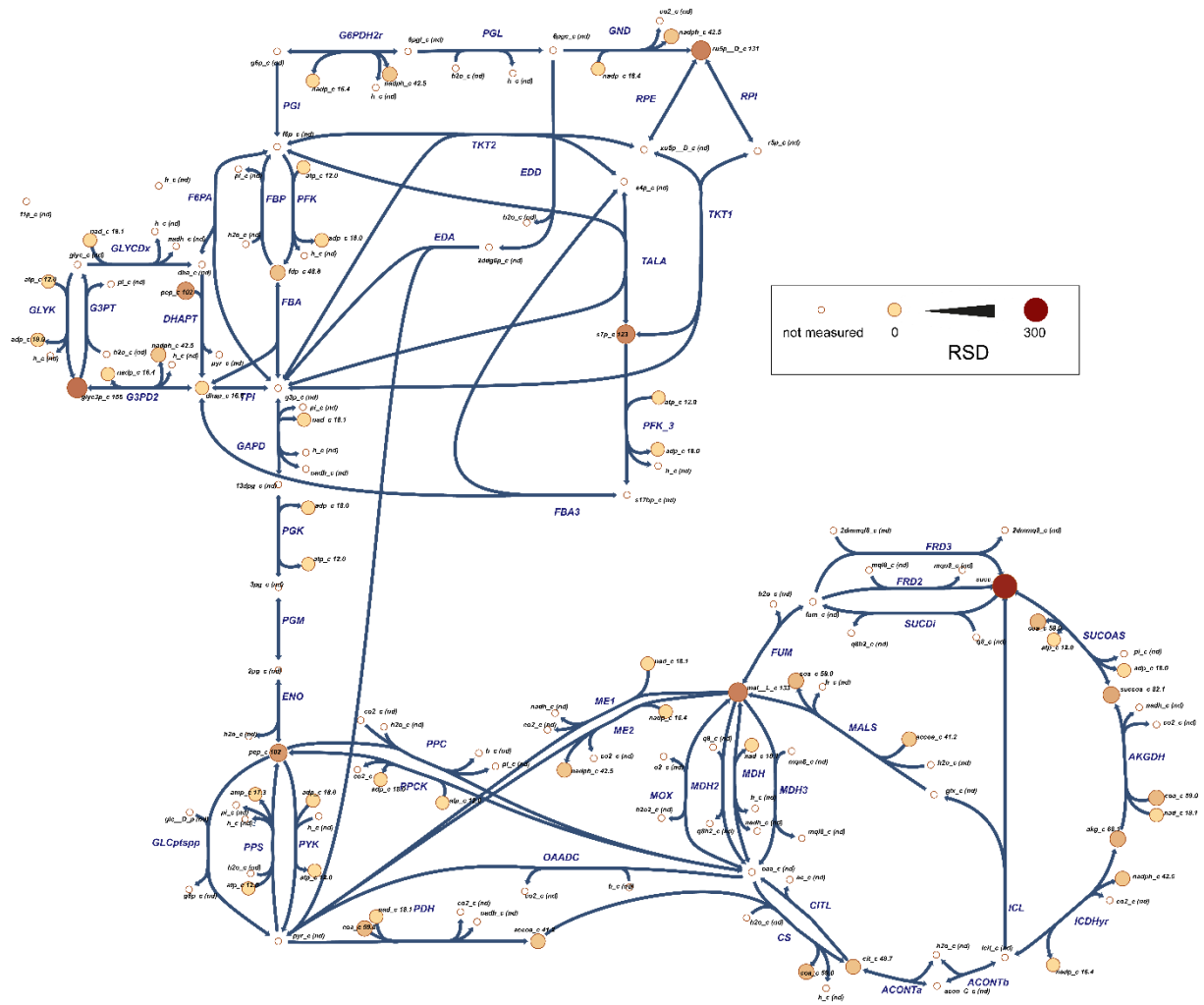
Figure S2. Relative standard deviations (RSD) of 101 metabolites (dots). Shown is the mean RSD in 19 conditions. The RSD is the standard deviation from n = 3 samples for each condition normalized to the mean in this condition. The boxplot shows the median (red), the box is the 0.75 and 0.25 quantile. Related to Figure 1.
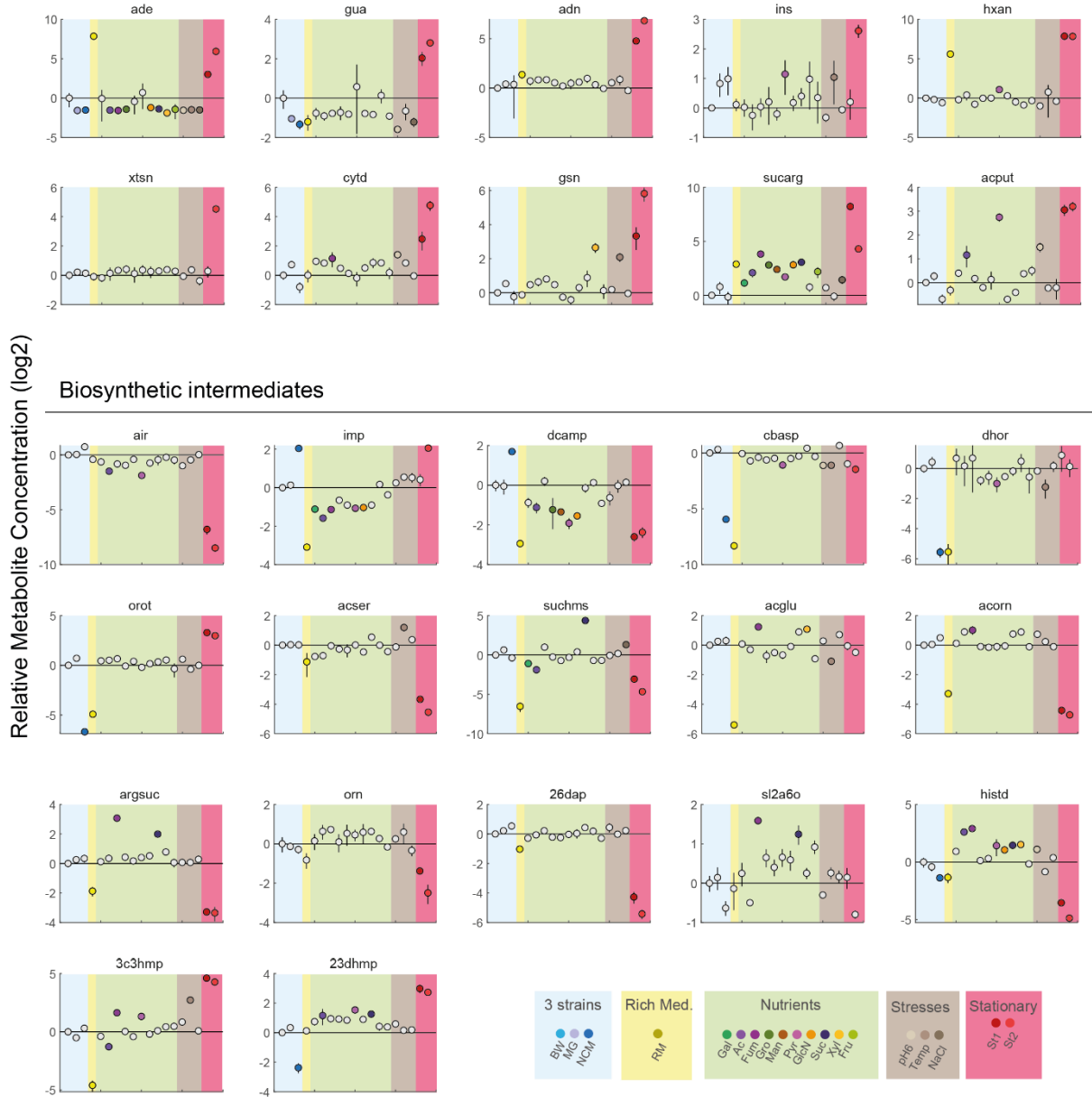
Figure S3. Relative concentration of metabolites that are degradation products of amino acids or nucleotides, and intermediates in biosynthetic pathways. Metabolite concentrations are normalized to the glucose condition with *E. coli* BW25113. Dots show the mean of n = 3 replicates and lines are the standard deviation. Conditions with log2 fold-changes >1 or <-1 are shown in the respective color. Abbreviations of metabolites are listed in Table S2. Related to Figure 1.

Figure S4. Variability of metabolites in central metabolism. The different sizes and colors of the dots represent the relative standard deviation (RSD) of metabolites across 16 of the 19 conditions (stationary phase and rich medium not considered). The RSD is the standard deviation across conditions normalized to the mean. Metabolites that were not measured are represented as smaller white dots. The figures were created with Escher (https://escher.github.io). Related to Figure 2.
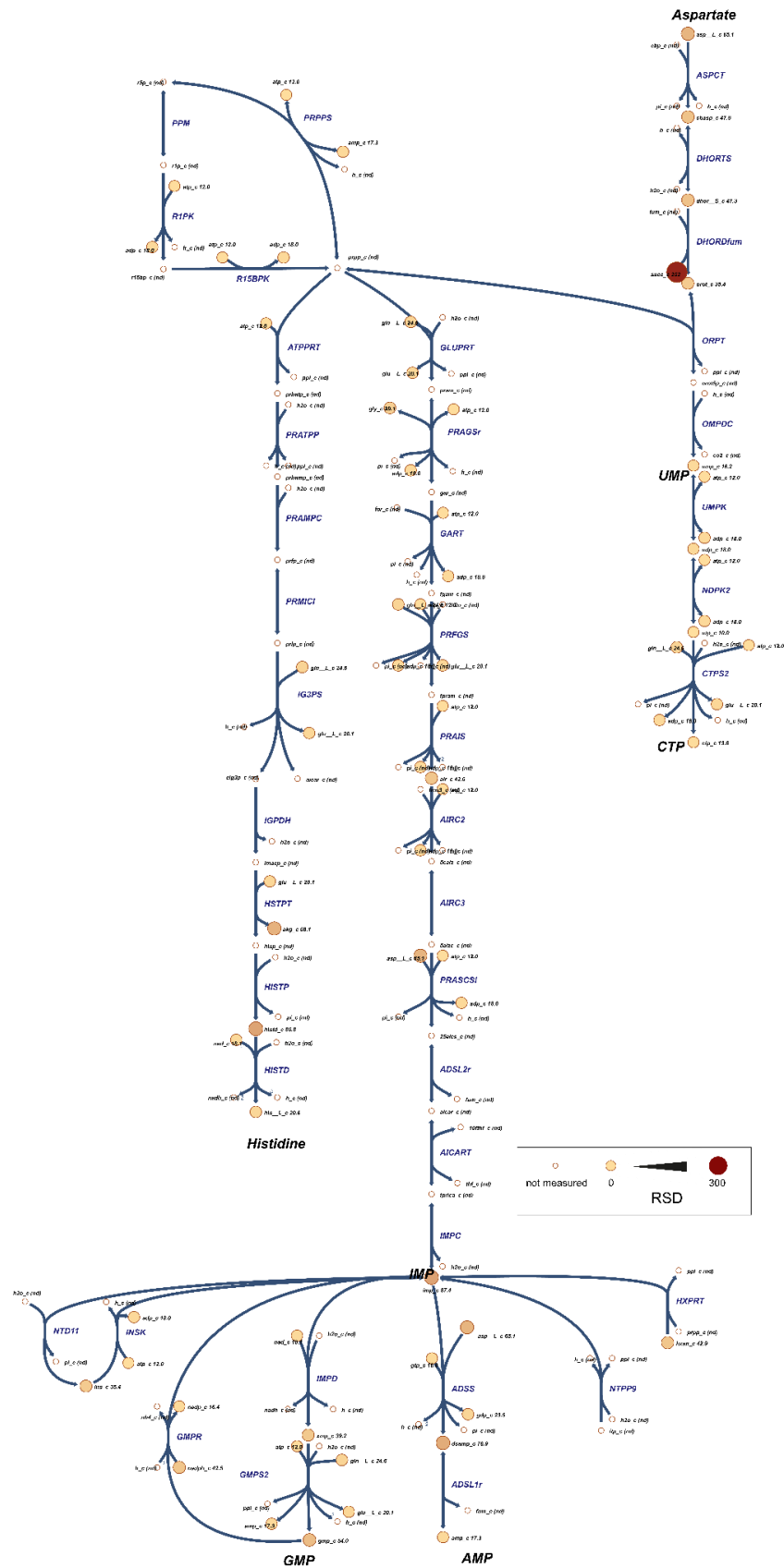
Figure S5. Variability of metabolites in nucleotide metabolism. The different sizes and colors of the dots represent the relative standard deviation (RSD) of metabolites across 16 of the 19 conditions (stationary phase and rich medium not considered). Related to Figure 2.
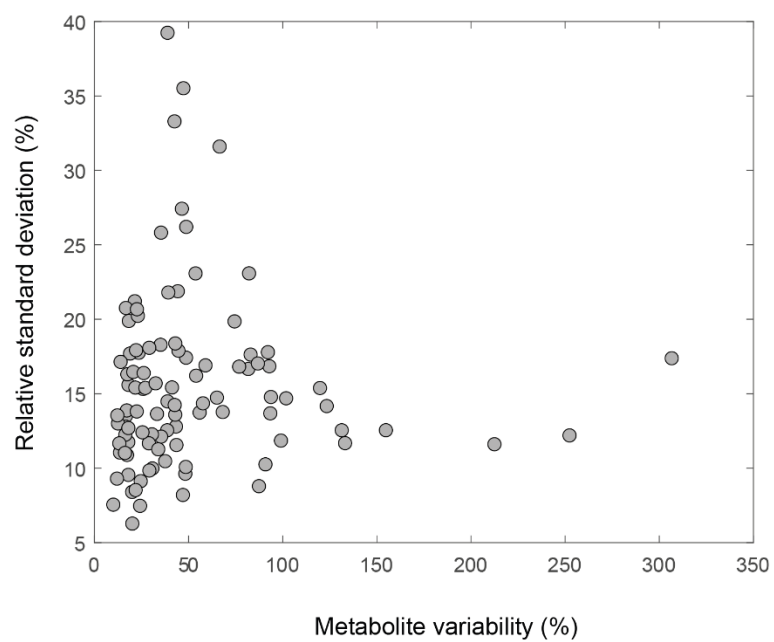
Figure S6 Relative standard deviations (RSD) of 101 metabolites (see Figure S2) is plotted against the metabolite variability. Related to Figure 2.
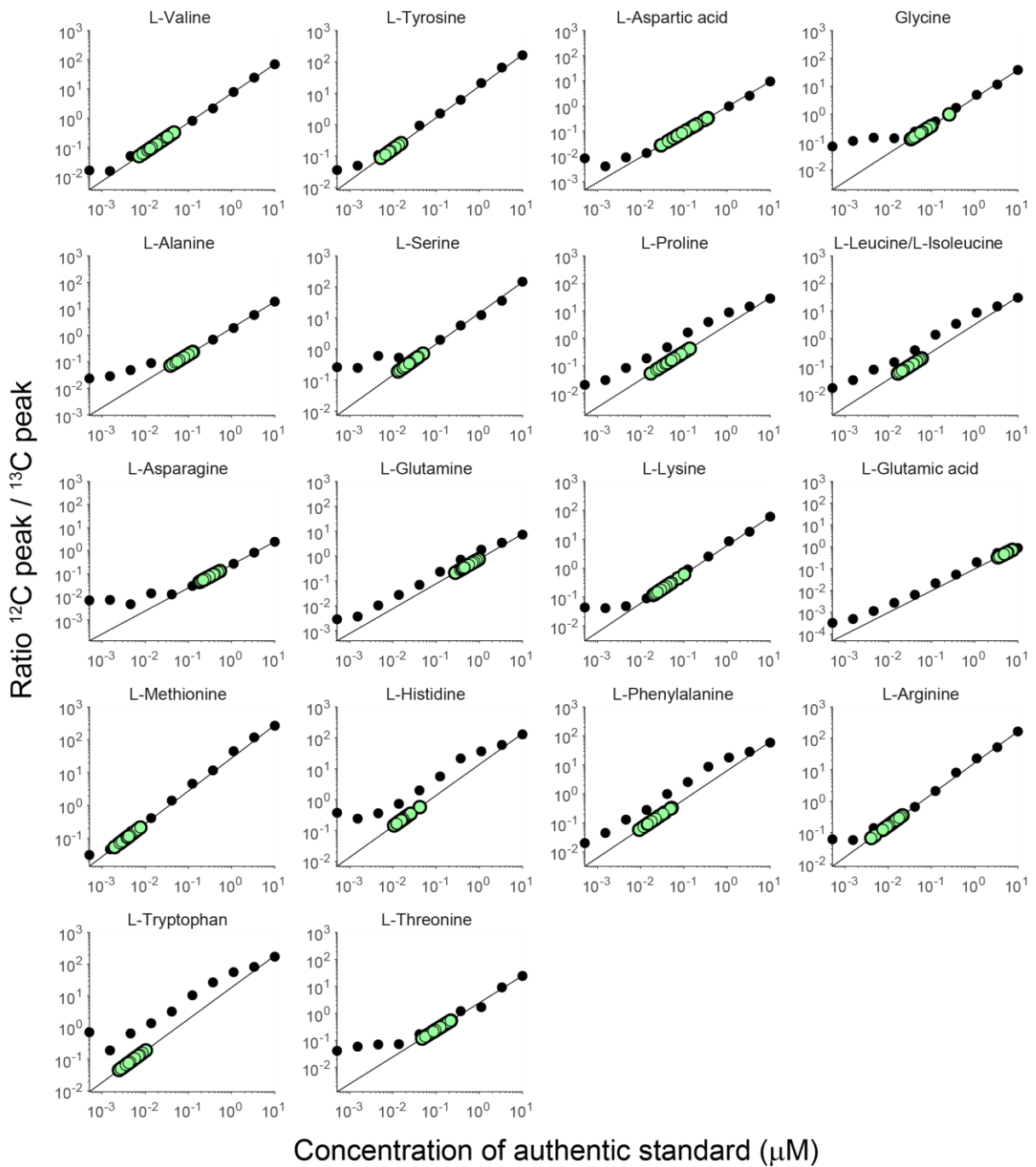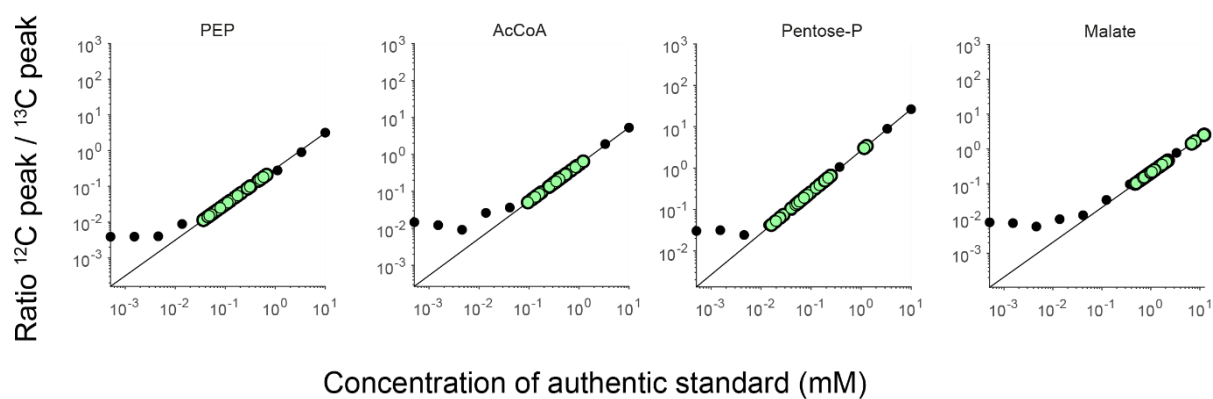
Figure S7 Response curves were measured with authentic standards of amino acids. The respective standard was measured at 10 different concentration levels with LC-MS/MS (black dots) and the 12C/13C ratios were fitted by linear regression (black line). Samples are shown in green. Related to Figure 2.
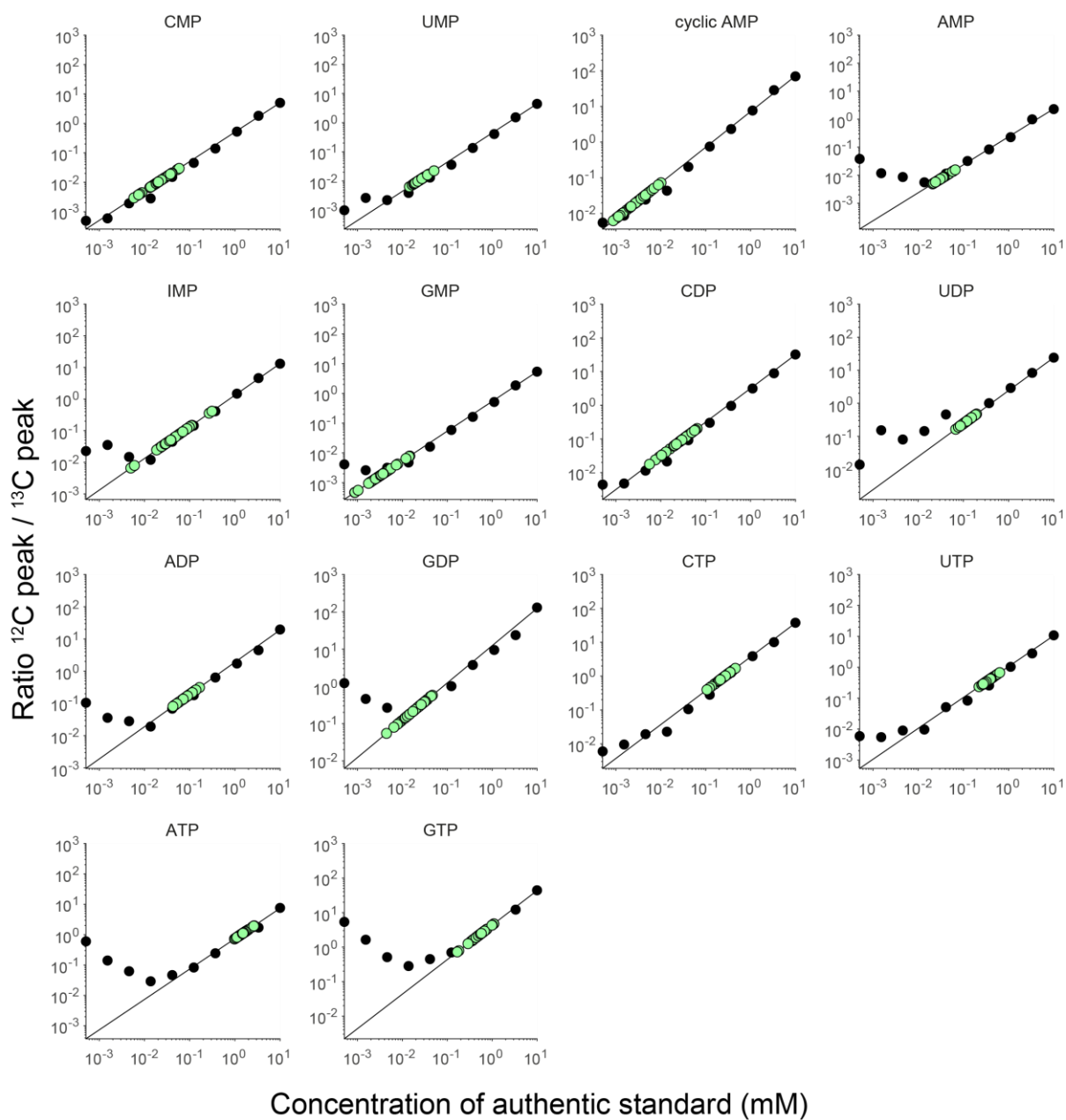
Figure S8 Response curves were measured with authentic standards of nucleotides. The respective standard was measured at 10 different concentration levels with LC-MS/MS (black dots) and the 12C/13C ratios were fitted by linear regression (black line). Samples are shown in green. Related to Figure 2.

Figure S9 Response curves were measured with authentic standards of phosphoenolpyruvate, acetyl-CoA, pentose-phosphates and malate. The respective standard was measured at 10 different concentration levels with LC-MS/MS (black dots) and the 12C/13C ratios were fitted by linear regression (black line). Samples are shown in green. Related to Figure 2.
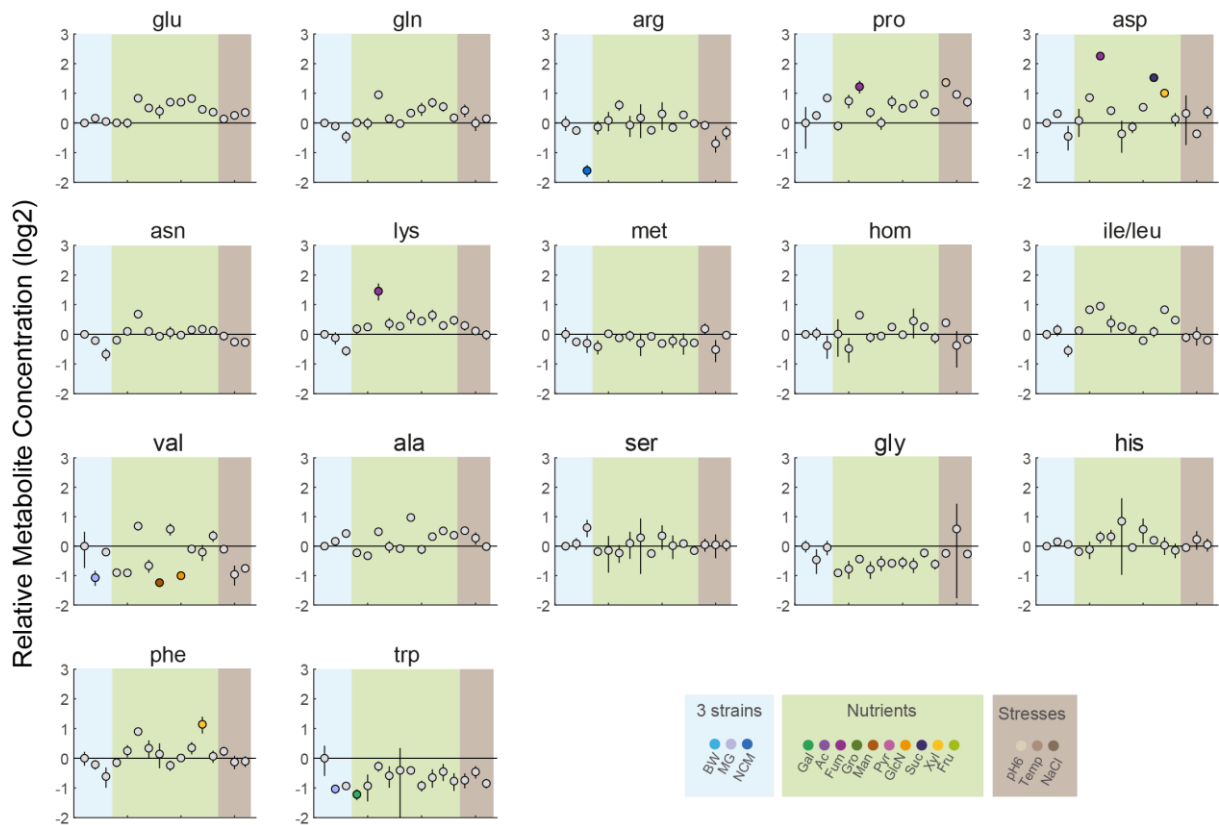
Figure S10 Relative concentration of amino acids. Amino acid concentrations are normalized to the glucose condition with *E. coli* BW25113. Dots show the mean of n = 3 replicates and lines are the standard deviation. Conditions with log2 fold-changes >1 or < -1 are shown in the respective color. Related to Figure 4.