Electronic Supplementary Material (ESI) for Molecular BioSystems

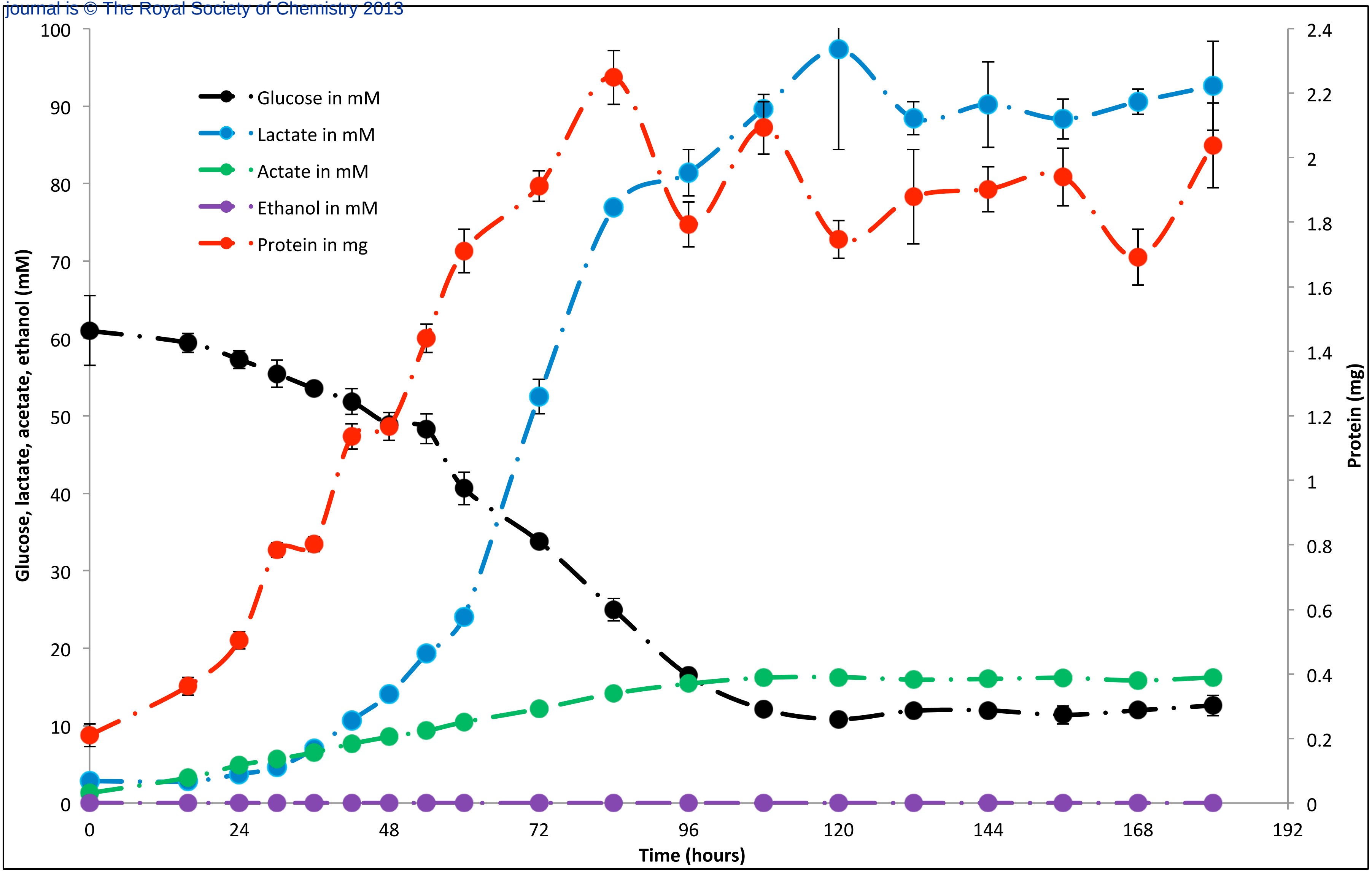

Figure S2. Glucose consumption and metabolite production of *M. pneumoniae* grown in batch culture. Data points are measured from biological triplicates.

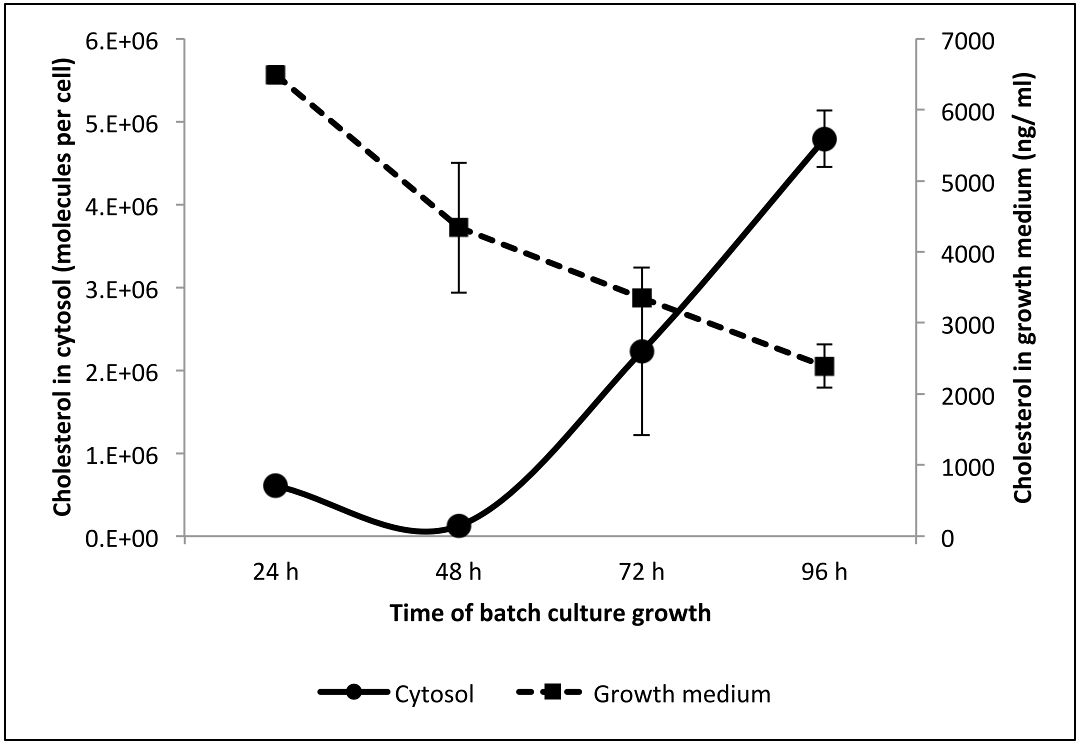

Figure S3. Cholesterol abundance in rich growth medium and in the bacterial cytosol during growth in batch culture.

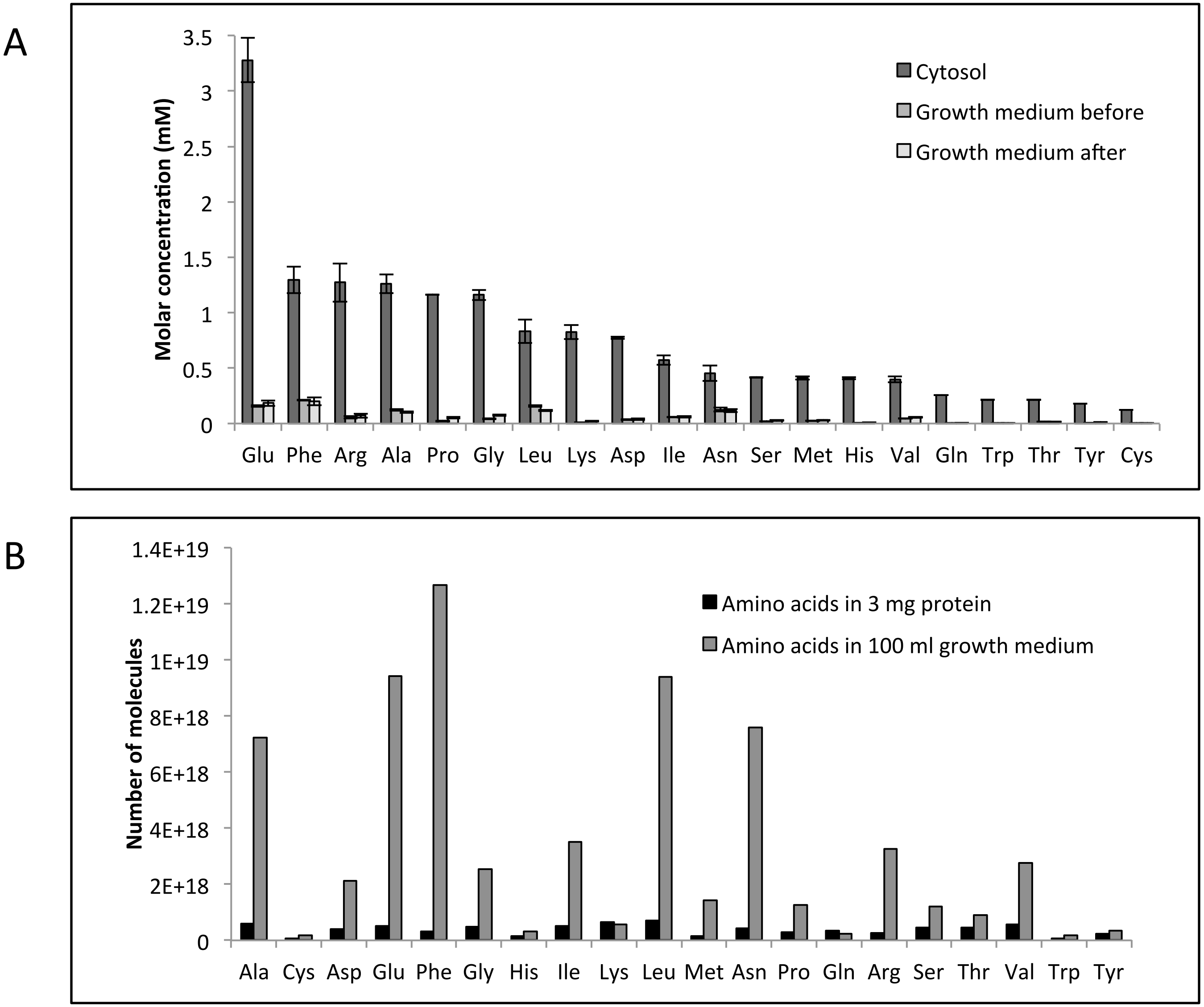

Figure S4. A: amino acid concentrations in the cytosol are higher than in the growth medium suggesting active import. B: Amino acids are not growth limiting.

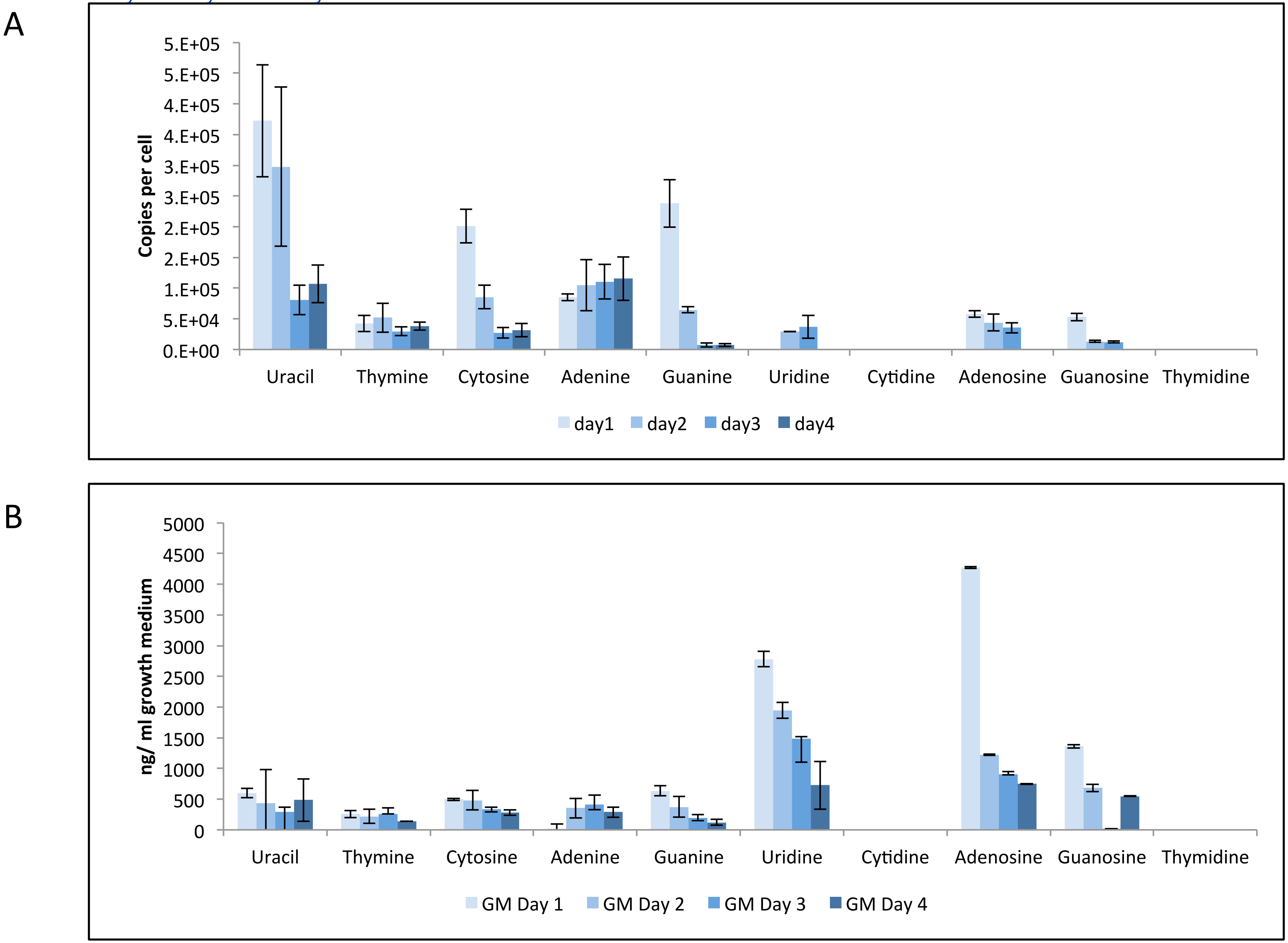

Figure S5. Measured free bases and nucleosides from the *M. pneumoniae* cytosol (A) and the growth medium (B) during four day batch culture growth.

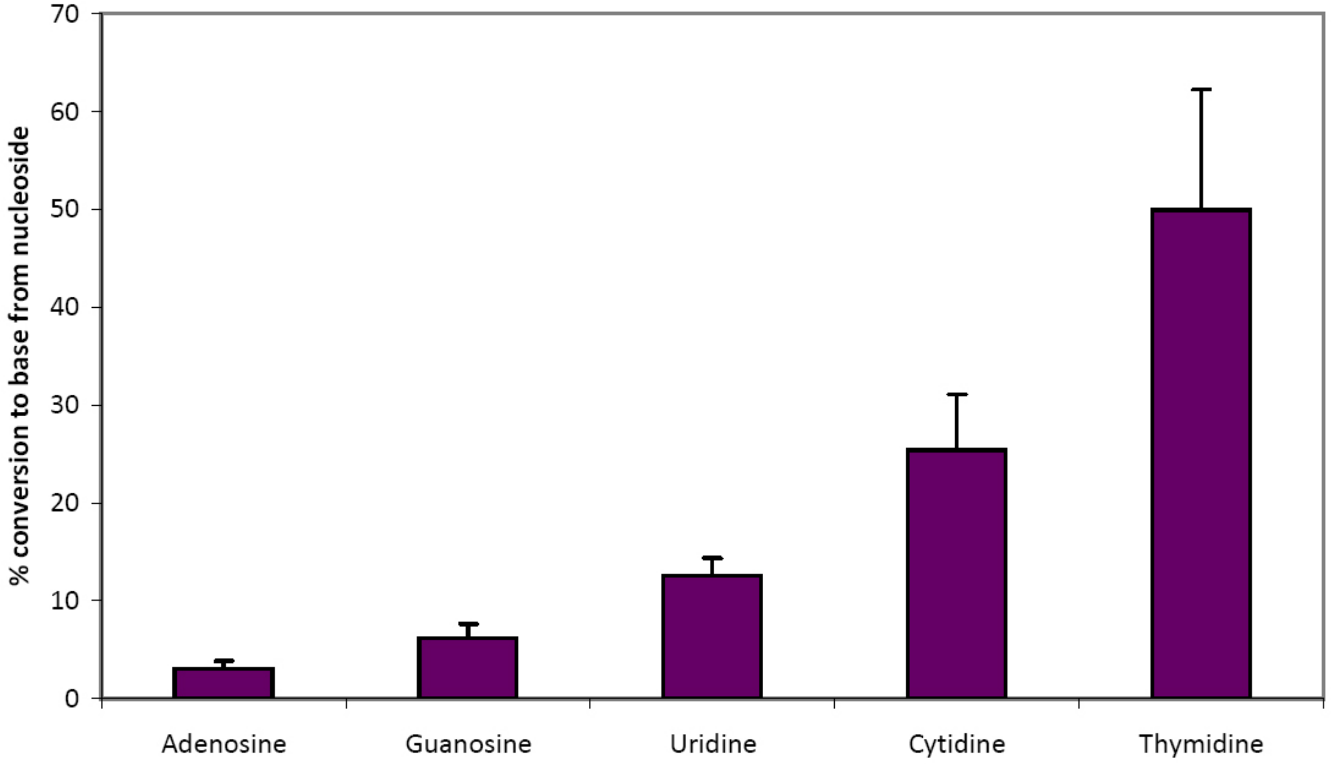

Figure S6. Conversion of nucleosides to nucleobases during sample preparation

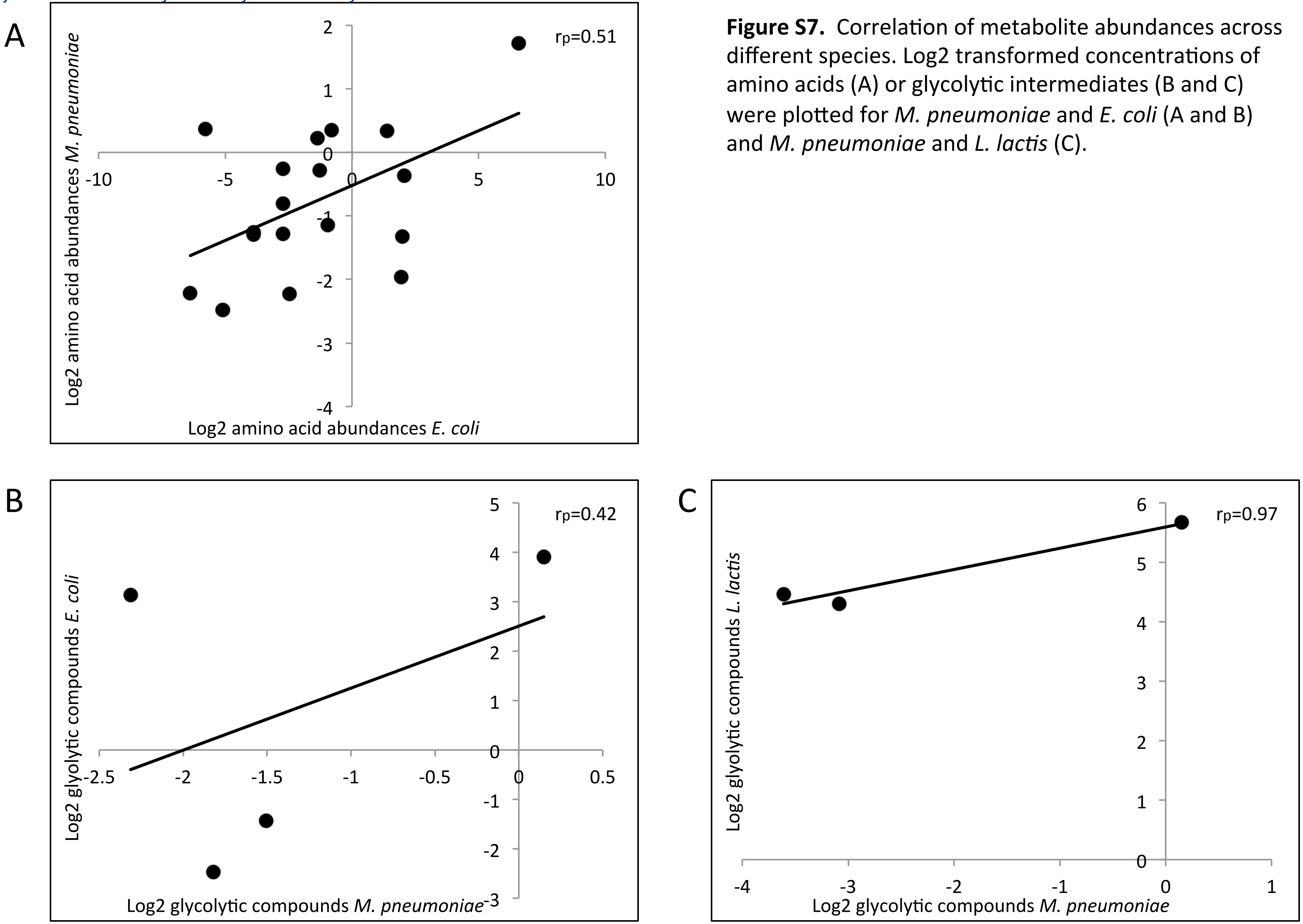

Figure S7. Correlation of metabolite abundances across different species. Log2 transformed concentrations of amino acids (A) or glycolytic intermediates (B and C) were plotted for *M. pneumoniae* and *E. coli* (A and B) and *M. pneumoniae* and *L. lactis* (C).