

	Amino acid concentration (mM)	
	AA medium	Biomass (1.8 g L ⁻¹)
Alanine	1.07	1.06
Arginine	0.57	0.53
Asparagine	0.43	0.46
Aspartate	1.09	0.41
Cysteine	0.08	0.06
Glutamine	1.65	0.32
Glutamate	0.34	0.32
Glycine	1.49	0.90
Histidine	0.19	0.17
Isoleucine	0.43	0.43
Leucine	0.79	0.80
Lysine	0.56	0.71
Methionine	0.19	0.21
Phenylalanine	0.31	0.32
Proline	0.37	0.38
Serine	0.51	0.47
Threonine	0.53	0.51
Tryptophan	0.10	0.10
Tyrosine	0.29	0.28
Valine	0.63	0.64

Table S1. Amino acid concentrations in AA medium and in *E. coli* K-12 MG1655 at biomass concentration 1.8 gram dry cell weight (gDCW) L⁻¹ (Valgepea et al. 2010). The amino acid concentrations in the culture broth were calculated based on the measured biomass concentration in culture broth (1.8 gDCW L⁻¹) and amino acid concentrations in the biomass of *E. coli* K-12 MG1655 at $\mu = 0.5$ h⁻¹. The AA medium was designed to result in similar amino acid concentrations with the exception of glutamate, glycine and aspartate since the target biomass concentration was the same (1.8 gDCW L⁻¹).

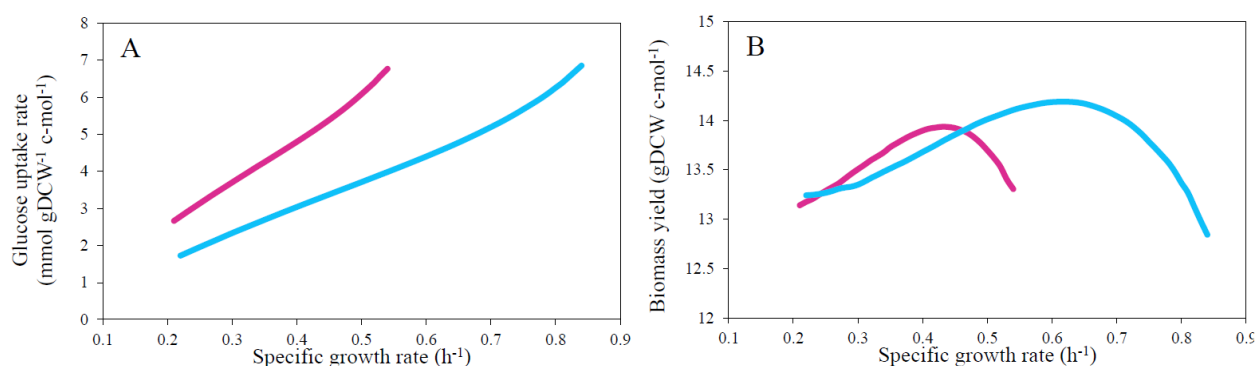


Fig. S1 Specific growth rate-dependent specific glucose uptake rate and biomass yield. A) Specific glucose uptake rate; B) Biomass yield. gDCW – gram of dry cellular weight. Biomass yield is shown in gDCW C-mol⁻¹ (gram of dry cellular weight produced from one mole of substrate carbon) units to enable comparison between AA and MG media. MG medium is represented in pink colour and AA medium in light blue.

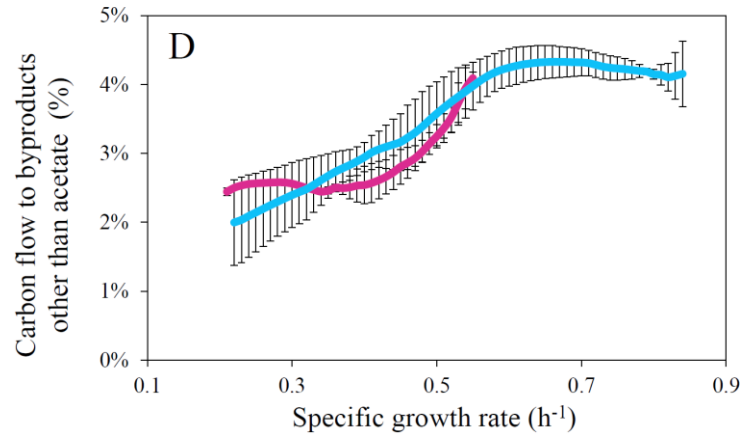


Fig. S2 μ -dependent sum carbon flow to by-products other than acetate. gDCW – gram dry cellular weight. MG medium is represented in pink colour and AA medium in light blue. Line is average of best-fit splines of 7–19 biomass and product concentration measurements in single experiments. Error bars represent the standard deviation between two biological replicates.

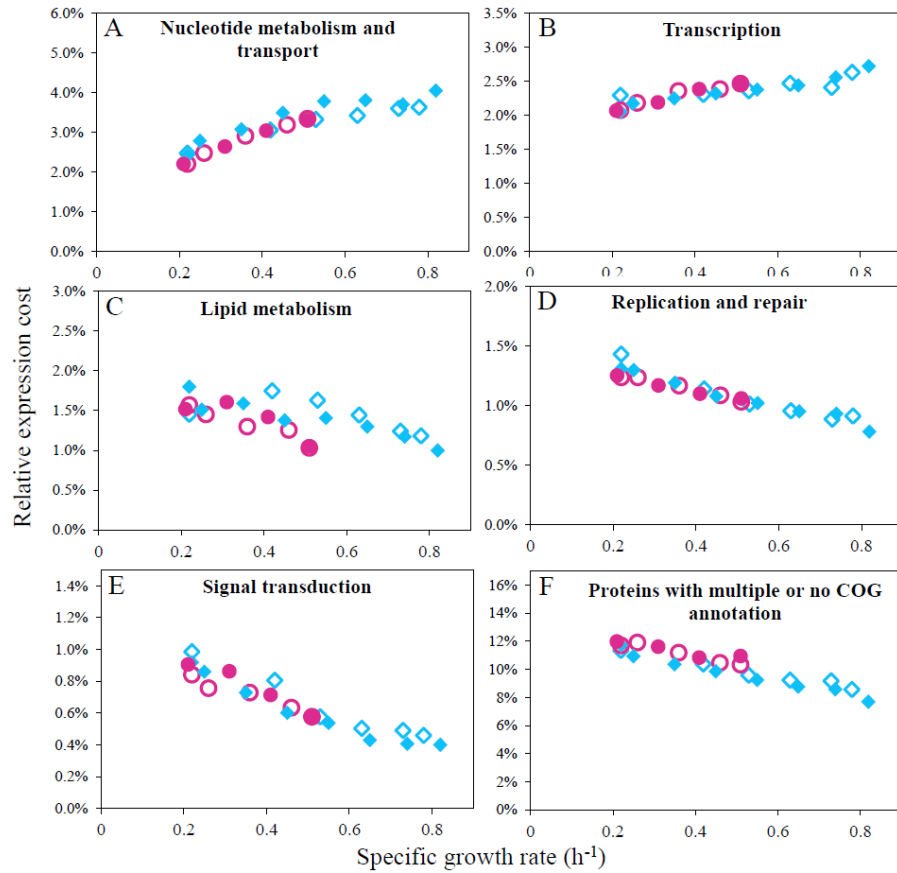


Fig. S3 Specific growth rate-dependent proteome expression cost allocation. Relative protein expression cost (compared to total expression cost) of COG groups A) “Nucleotide metabolism and transport” (COG group F) B) “Transcription” (COG group K) C) “Lipid metabolism” (COG group I) D) “Replication and repair” (COG group L) E) “Signal transduction” (COG group T) F) “Proteins with multiple or no COG annotation”. MG medium depicted with pink circles and AA medium with light blue diamonds, open and solid shapes represent different biological replicates.