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Supplemental Information

Allosteric Feedback Inhibition Enables Robust Amino Acid Biosynthesis in *E. coli* by Enforcing Enzyme Overabundance

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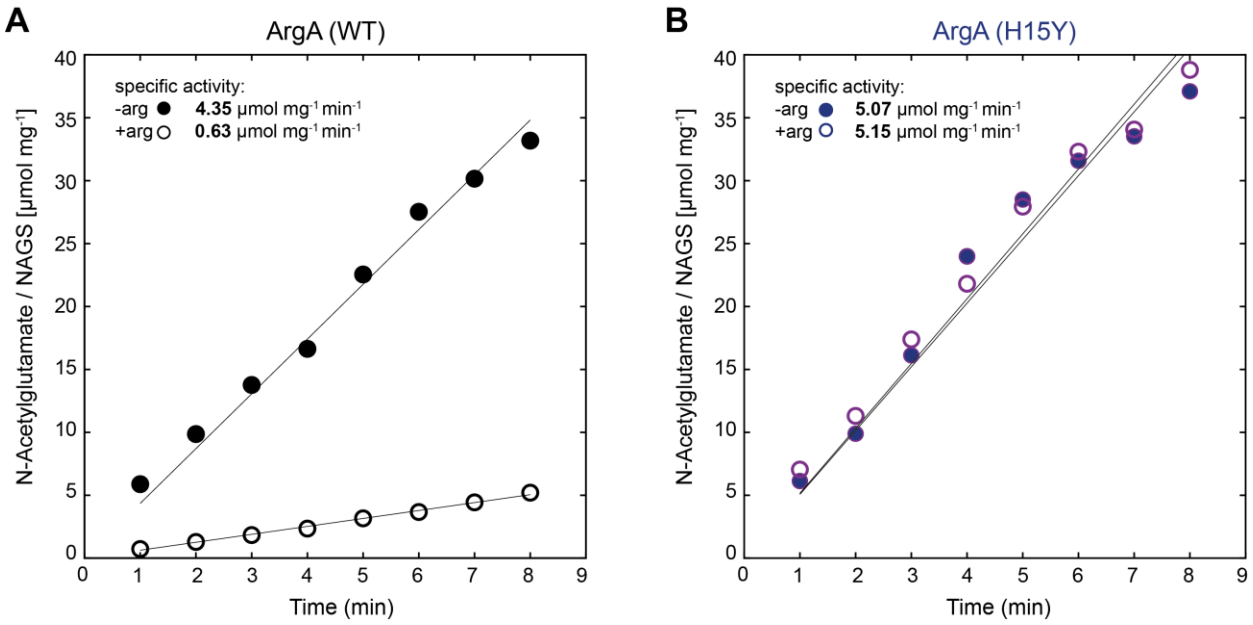

Figure S1. Related to Figure 1; *In vitro* kinetics of N-acetylglutamate-synthase (NAGS) from *E. coli* (ArgA) in the **A** native and **B** the allosteric feedback resistant version ArgA (H15Y). Dots represent means from n=2 independent assays (filled = no arginine; empty = 1 mM arginine). Activity of His-tagged purified enzymes was assayed in 30 mM TRIS buffer (40 mM L-glutamate, 0.65 mM Acetyl-CoA and 10 mM $MgCl_2$). For sampling 10 µL of reaction solution was transferred into 40 µL of 50:50 (v-%) acetonitrile/methanol at -20°C. The reaction product N-acteylglutamate was measured by LC-MS/MS. Specific activity in [µmol mg^{-1} min^{-1}] was calculated from linear regression through the 8 time points.

Figure S2. Related to Figure 1; Relative concentrations of 110 intracellular metabolites in wild-type *E. coli* and seven dysregulated mutants (n = 3).

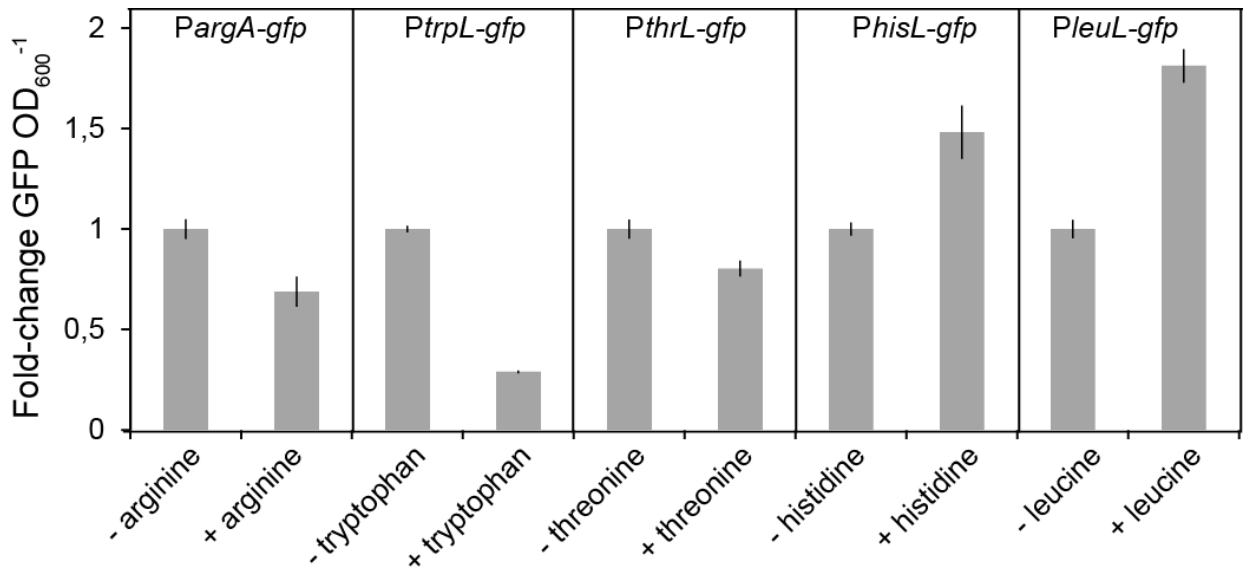

Figure S3. Related to Figure 2; GFP expression of promotor fusions P*argA-gfp*, P*trpL-gfp*, P*thrL-gfp*, P*hisL-gfp* and P*leuL-gfp* in *E. coli* wild-type with and without addition of external amino acids. Bar plots show fold-changes of GFP per OD_{600} relative to the condition without external amino acids (n=3). Cells were grown in M9 minimal medium (5 g L^{-1} glucose) and GFP expression was measured in mid-exponential phase at OD_{600} ~0.5 with a plate reader. Amino acids were supplemented to a final concentration of 2 mM.

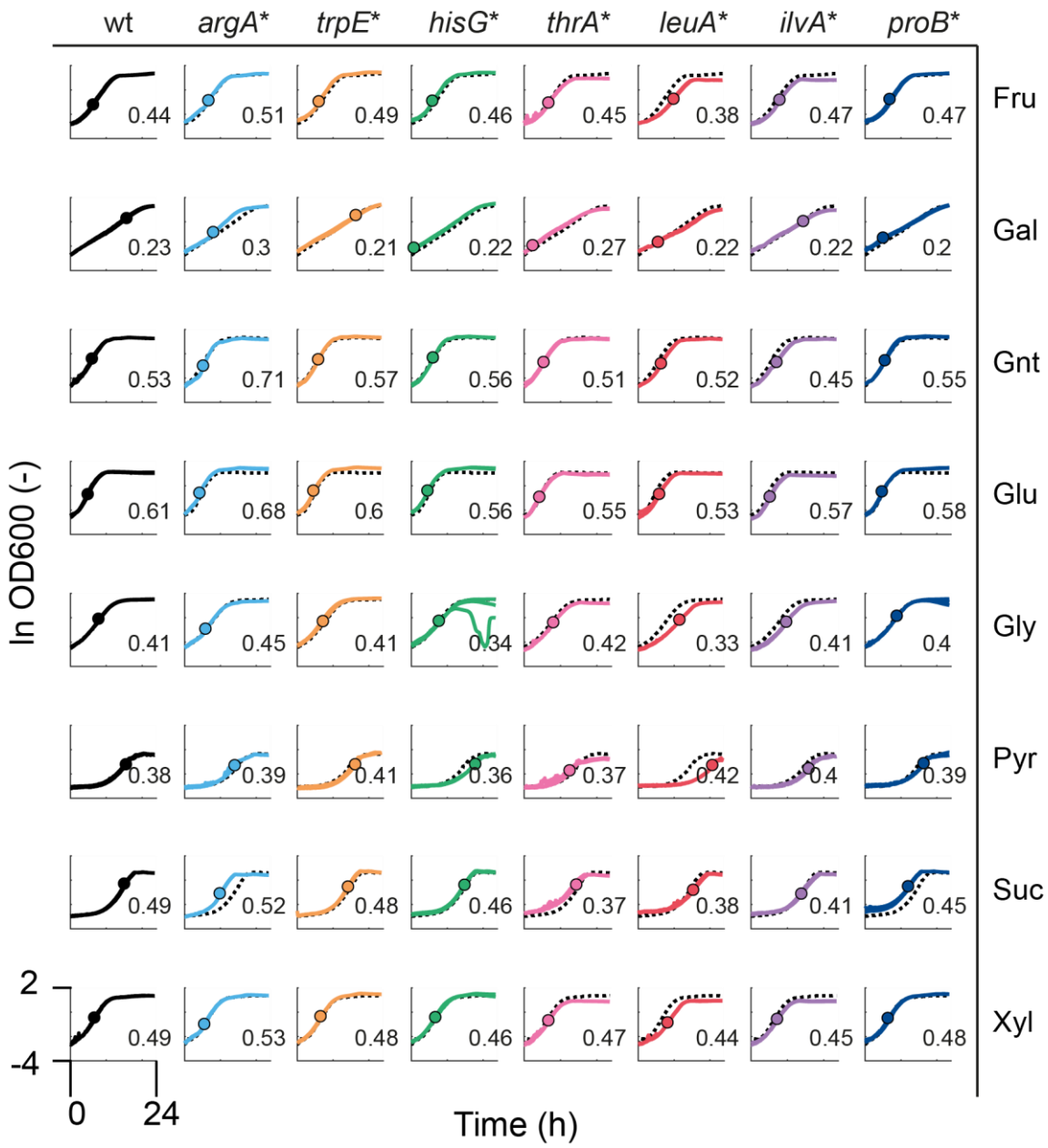

Figure S4. Related to Figure 3; Growth of wild-type *E. coli* and 7 mutants (see also Figure 1A) on fructose (Fru), galactose (Gal), gluconate (Gnt), glucose (Glu), glycerol (Gly), pyruvate (Pyr), succinate (Suc), and xylose (Xyl). Shown are three cultivations in microtiter plates. The dashed line is the mean of the wild-type in the particular condition (n = 3). Numbers are the maximal growth rates in h^{-1}, which is reached at the time indicated by dots. All x-axes range from 0 to 24 hours. All y-axes range from -4 to 2 ($lnOD_{600}$).

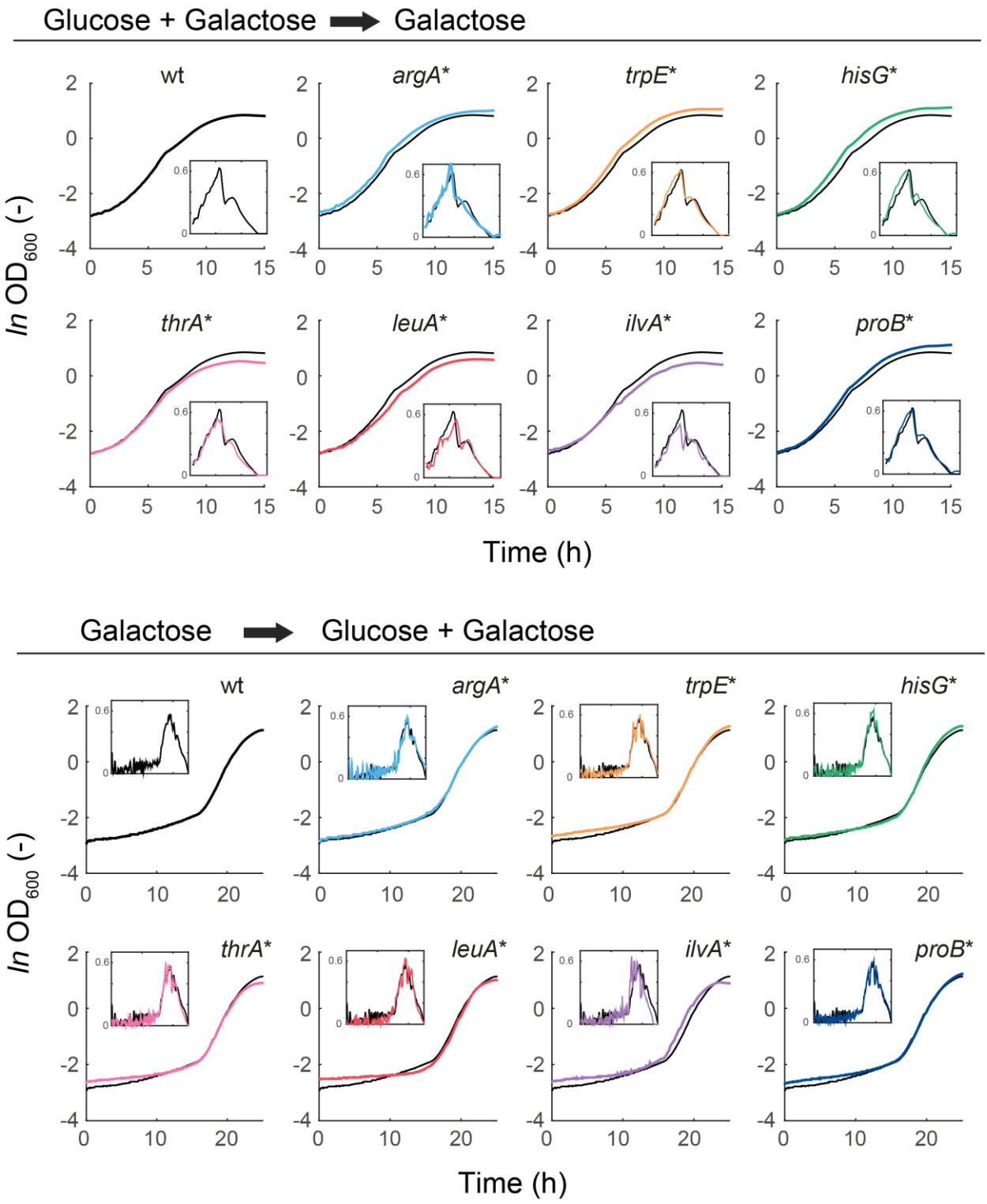

Figure S5. Related to Figure 3; Growth of wild-type *E. coli* and the seven dysregulated mutants in shifts between glucose and galactose. For down-shifts from glucose to galactose, cells were grown in M9 minimal medium with 0.5 g L^{-1} glucose and 5 g L^{-1} galactose. For up-shifts from galactose to glucose, cells were grown in M9 minimal medium with 5 g L^{-1} galactose and glucose was added to a final concentration of 5 g L^{-1} at an OD of 0.1. Shown are means of n = 3 cultures. Inserts show the growth rate during the same time period. Growth rates were estimated by linear regression over a moving 30 minute window. The same wild-type growth curve is shown in each graph in black as a reference.

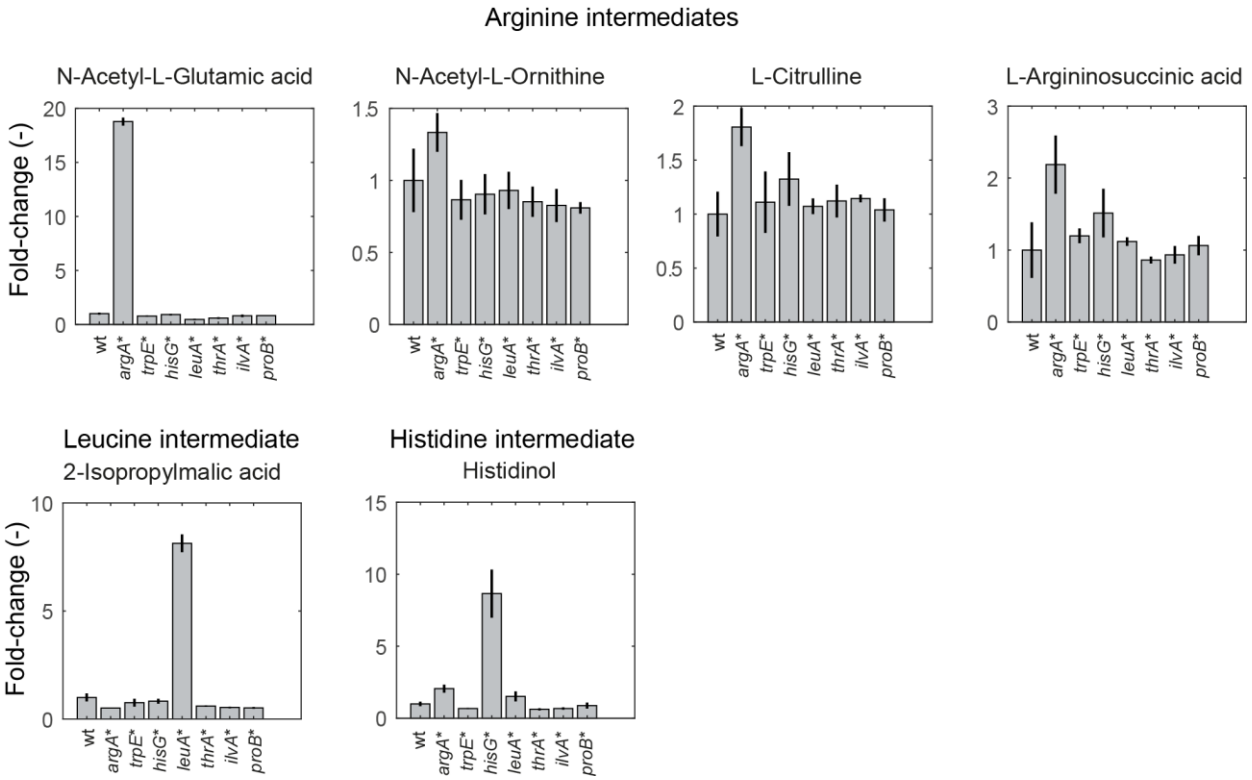

Figure S6. Related to Figure 4; Intermediates in dysregulated pathways measured by LC-MS in wild-type *E. coli* and seven dysregulated mutants (n = 3).

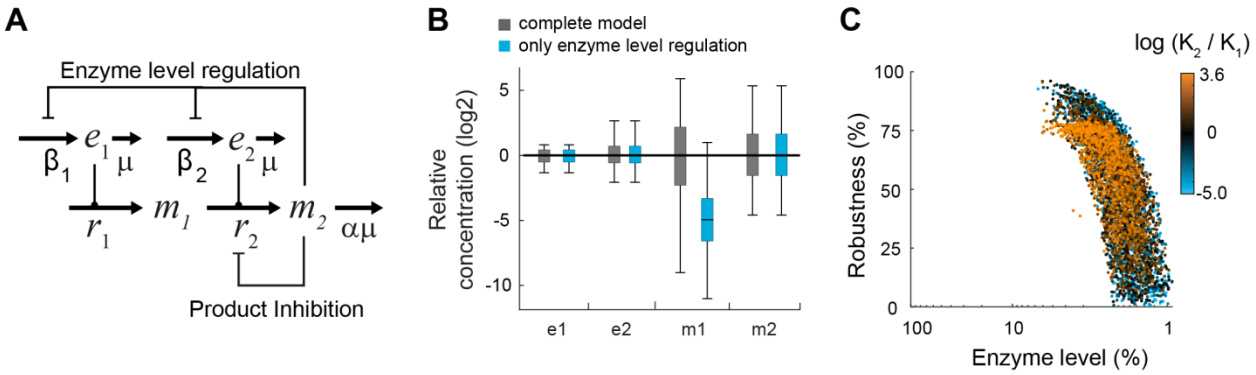

Figure S7. Related to Figure 4;

(A) Model with product inhibition, instead of allosteric feedback inhibition. Metabolite 2 inhibits reaction 2 by competitive product inhibition, which was modelled using the following equation:

$$r_2 = k_{cat,2} \cdot e_2 \cdot \frac{m1}{m1 + Km \cdot \left(1 + \frac{m2}{K1}\right)}$$

(B) Steady state concentrations of e_1, e_2 m_1 and m_2 calculated with 5000 simulations for the complete model (grey), and the model with only enzyme level regulation (blue). Boxes contain 50% and whiskers 99% of the simulated concentrations. All concentrations are normalized to the median concentrations of the complete model.

(C) Enzyme levels (sum of e_1 and e_2) and robustness against perturbations of $\beta_{2,max}$ for 5000 simulations of the complete model (dots). The color of each dot shows the ratio of inhibition constants for allosteric feedback inhibition (K1) and enzyme level regulation (K2) in the respective model. Robustness corresponds to the percentage downregulation of $\beta_{2,max}$ that was tolerated by each model. 100% enzyme abundance corresponds to the maximum theoretical enzyme concentration in the model.

Table S1. Related to Figure 1; Mutations in allosteric enzymes that were investigated in this study.

Pathway	Gene	Enzyme	Mutation	Reference
L-arginine biosynthesis	*argA*	N-acetylglutamate synthase	H15Y	Rajagopal et al., 1998
L-isoleucine biosynthesis	*ilvA*	Threonine deaminase	L447F	LaRossa et al., 1987
L-histidine biosynthesis	*hisG*	ATP phosphoribosyl transferase	E271K	Doroshenko et al., 2013
L-leucine biosynthesis	*leuA*	2-isopropylmalate synthase	G462D	Gusyatiner et al., 2002
L-proline biosynthesis	*proB*	Glutamate-5-kinase	D107N	Csonka et al., 1988
L-threonine biosynthesis	*thrA*	Aspartate kinase	S345F	Lee et al., 2003
L-tryptophan biosynthesis	*trpE*	Anthranilate synthase	S40F	Caligiuri and Bauerle, 1991

Table S2. Related to Figure 1; Oligonucleotides for recombineering

Gene	Oligonucleotides for recombineering (5'-3')	Protospacer sequence (5'-3')
argA	GTGGTAAAGGAACGTAAAACCGAGTTGGTCGAGGGAT TCCGCTATTCAGTTCCCTATATCAATACCCACCGGGGAA	GGTCGAGGGATT CCGCCATT
ilvA	GGAATCACCGGGCGCGTTCCTGCGCTTTCTCAACACG CTGGGTACGTACTGGAACATTTCTTTGTTCCACTATCG	CAACACGCTGG GTACGTACT
hisG	GTCAGCAGCAAAACCCTGTTCTGGGAAACTATGGAAA AACTGAAAGCGCTGGGGCCAGTTCAATTCTGGTCCTG	TGGAAAAACTGA AAGCGCTG
leuA	CTGGTGAAATACAGCCTGACCGCCAAAGGACACGGTA AAGATGCGCTGGATCAGGTGGATATCGTCGCTAACTAC	CGGTAAAGATGC GCTGGGTC
proB	ACCCGTGCTAATATGGAAGACCGTGAACGCTTCCTGAACG CTCGCGACACCCTGCGAGCGTTGCTCGATAACAATATC	CGACACCCTGCG AGCGTTGC
thrA	GCGCGCGTCTTTGCAGCGATGTCACGCGCCCGTATTT TCGTGGTGCTGATTACGCAATCATCTTCCGAATACAGC	TGGTGCTGATTA CGCAATCA
trpE	CTTATCGCGACAATCCCACTGCGCTTTTTCACCAGTTGTGT GGGGATCGTCCGGCAACGCTGCTGCTGGAATTCGCAGAT	CGCTTTTTCACC AGTTGTGT

Table S3. Related to Figure 4; Literature k_{cat} values for enzymes in amino acid biosynthesis. The values were collected from the BRENDA database, and from Davidi and Milo, 2017. - indicates that no value could be found in both sources. The 25th and 75th quartiles of these k_{cat} values are 930 min^{-1} and 4140 min^{-1}, respectively.

Name	k_{cat}, s^{-1}	Name	k_{cat}, s^{-1}	Name	k_{cat}, s^{-1}
argA	654.00	cysK	378.50	ilvN	40.00
argB	-	cysM	24.00	leuA	-
argC	-	cysN	-	leuB	69.00
argD	-	cysQ	11.00	leuC	-
argE	1800.00	dadX	33.66	leuD	-
argF	-	dapA	104.00	lysA	33.00
argG	-	dapB	382.00	lysC	22.13
argH	-	dapD	36.00	metA	22.00
argI	-	dapE	-	metB	121.00
aroA	32.00	dapF	84.00	metC	34.10
aroB	14.00	gdhA	37.00	metE	3.50
aroC	39.00	glnA	33.00	metH	-
aroD	75.00	gltB	-	metL	-
aroE	237.00	gltD	-	pheA	32.00
aroF	-	glyA	10.00	proA	10.00
aroG	4.20	hisA	7.20	proB	53.00
aroH	-	hisB	-	proC	717.00
aroK	-	hisC	-	prs	-
aroL	-	hisD	12.00	serA	29.00
asd	-	hisF	-	serB	-
asnA	-	hisG	-	serC	1.80
asnB	4.50	hisH	-	thrA	-
aspC	-	hisI	-	thrB	17.00
avtA	-	ilvA	-	thrC	-
cysC	50.00	ilvB	38.50	trpA	-
cysD	-	ilvC	0.30	trpB	-
cysE	772.00	ilvD	69.00	trpC	18.77
cysH	-	ilvE	-	trpE	-
cysI	47.00	ilvH	-	tyrA	71.00
cysJ	-	ilvI	-	tyrB	-

Table S4. Related to Figure 4; Amino acid requirements of *E. coli* (Monk et al., 2017). The mean of 86.6 mM was used as parameter α in the model.

Amino Acid	Coefficients, mmol g_{dw}^{-1}	alpha, mM
ala-L	0.499	166.4
arg-L	0.287	95.8
asn-L	0.234	78.1
asp-L	0.234	78.1
cys-L	0.089	29.7
gln-L	0.256	85.2
glu-L	0.256	85.2
gly	0.595	198.4
his-L	0.092	30.7
ile-L	0.282	94.1
leu-L	0.438	145.9
lys-L	0.333	111.1
met-L	0.149	49.8
phe-L	0.180	60.0
pro-L	0.215	71.6
ser-L	0.210	69.9
thr-L	0.247	82.2
trp-L	0.055	18.4
tyr-L	0.134	44.7
val-L	0.411	137.1
Mean	**0.260**	**86.6**

Table S5. Related to Figure 4; Inhibition constants of allosteric enzymes (K_i-value), transcriptional attenuation (tRNA-ligase K_m-value) and metabolite-transcription factor interactions (K_d-value). Values were obtained from EcoCyc (Keseler et al., 2017), Brenda (Schomburg et al., 2002) or RegulonDB (Gama-Castro et al., 2016). When more than one value was available, an upper and a lower bound are given. The grey background indicates the seven pathways that were investigated during this work. The Ki of ArgA was measured in this work with *in vitro* assays.

Biosynthesis pathway	Allosteric Feedback		K_i mM		Transcriptional Feedback			$K_{m/d}$ mM	
	Enzyme	Metabolite	LB	UB	Mechanism	Protein	Metabolite	LB	UB
Arginine	ArgA	arg	0.15		Repressor	ArgR	arg	0.28	
Asparagine	AsnA	asn	0.12		Repressor	AsnC	asn	1	
Cysteine	CysE	cys	0.001						
Histidine	HisG	his	0.012	0.1	Attenuation	his-tRNA ligase	his	0.008	0.03
Isoleucine	IlvA	ile	0.06		Attenuation	ile-tRNA ligase	ile	0.0036	1.3
Leucine	LeuA	leu	0.28		Attenuation	leu-tRNA ligase	leu	0.0015	0.05
Lysine	DapA	lys	0.21	3.9					
Methionine	MetA	met	0.1	4	Repressor	MetJ	sam	0.01	0.05
Phenylalanine	PheA	phe	0.1	0.6		TyrR	phe	>0.18	
Proline	ProB	pro	0.02						
Serine	SerA	ser	0.005	0.37					
Threonine	ThrA	thr	0.097	0.167	Attenuation	thr-tRNA ligase	thr	0.11	0.2
Tryptophan	TrpE	trp	0.17		Repressor	TrpR	trp	0.16	
Tryptophan	TrpE	trp	0.17		Attenuation	trp-tRNA ligase	trp	0.017	
Tyrosine	TyrA	tyr	0.1		Repressor	TyrR	tyr	0.18	
Valine	IlvB	val	0.078	0.1	Attenuation	val-tRNA ligase	val	0.0043	0.1

Table S6. Related to STAR Methods; Oligonucleotides used in this study.

Oligonucleotide	Sequence (5'-3')	Description
argA_Forward	GGTCGAGGGATTCCGCCATTG TTTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against argA
argA_Reverse	AATGGCGGAATCCCTCGACCG TGCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against argA
ilvA_Forward	AGTACGTACCCAGCGTGTTGG TTTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against ilvA
ilvA_Reverse	CAACACGCTGGGTACGTACTG TGCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against ilvA
hisG_Forward	CAGCGCTTTCAGTTTTTCCAGT TTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against hisG
hisG_Reverse	TGGAAAAACTGAAAGCGCTGG TGCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against hisG
leuA_Forward	GACCCAGCGCATCTTTACCGG TTTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against leuA
leuA_Reverse	CGGTAAAGATGCGCTGGGTCG TGCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against leuA
proB_Forward	GCAACGCTCGCAGGGTGTCGG TTTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against proB
proB_Reverse	CGACACCCTGCGAGCGTTGCG TGCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against proB
thrA_Forward	TGATTGCGTAATCAGCACCAG TTTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against thrA
thrA_Reverse	TGGTGCTGATTACGCAATCAG TGCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against thrA
trpE_Forward	ACACAACTGGTGAAAAAGCGG TTTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against trpE
trpE_Reverse	CGCTTTTTCACCAGTTGTGTG TGCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against trpE
argR_Forward	ATTCTTCAATGGACTGGAGGG TTTTAGAGCTAGAAATAGCAAG	Forward primer used with **CPEC001** for amplification of fragment 1 for customzied pKDsgRNA targeted against argR
argR_Reverse	CCTCCAGTCCATTGAAGAATGT GCTCAGTATCTCTATCACTGA	Reverse primer used with **CPEC002** for amplification of fragment 2 for customzied pKDsgRNA targeted against argR
CPEC001	TTTATAACCTCCTTAGAGCTCGA	Reverse primer for amplification of fragment 1 for pKDsgRNA
CPEC002	CCAATTGTCCATATTGCATCA	Forward primer for amplification of fragment 2 for pKDsgRNA
Ec-F	GTTTTAGAGCTAGAAATAGCAA GTTAAAATAAGGC	Foward primer used with **guide_Rev** for ampflification of customized pNUT1533-ctrl
Ec-F-argE-mm5	TTTTTCATTGTTGACACCCCTC GTTTTAGAGCTAGAAATAGCAA GTTAAAATAAGGC	Foward primer used with **guide_Rev** for ampflification of customized pNUT1533-argE
Ec-F-trpA	TTCTTTGCGCTCCTTCAACTGTT TTAGAGCTAGAAATAGCAAGTT AAAATAAGGC	Foward primer used with **guide_Rev** for ampflification of customized pNUT1533-trpA
Ec-F-hisB	TCACTCGGCGGTTCGCTAATCA GTTTTAGAGCTAGAAATAGCAA GTTAAAATAAGGC	Foward primer used with **guide_Rev** for ampflification of customized pNUT1533-hisB
Ec-R	ACTAGTATTATACCTAGGACTG AGCTAGC	Reverse primer for amplification of customized pNUT1533 plasmids
ArgA_fwd_NdeI	TGACCATATGATGGTAAAGGAA CGTAAAAC	Amplification of genomic argA
ArgA_rev_BamHI	TGACGGATCCTTACCCTAAATC CGCCATCA	Amplification of genomic argA
ArgA_H15Y_fwd	AGGGAACCGAATAGCGGAATC CCTC	Forward primer for amplification pET28a(+)-argA
ArgA_H15Y_rev	ATATCAATACCCACCGGG	Reverse primer for amplification pET28a(+)-argA
hisL_fwd_gfp	CCGCTCGAGGCTTTCATCATTG TTGCCG	Forward primer for amplification of hisL attenuator region
hisL_rev_gfp	CCGGGATCCCGCAGAATATCAA TCGGC	Reverse primer for amplification of hisL attenuator region
leuL_fwd_gfp	CCGCTCGAGTTGTCCCCTTTTT CCTCG	Forward primer for amplification of leuL attenuator region

Table S6. Related to STAR Methods; continued

Oligonucleotide	Sequence (5'-3')	Description
leuL_rev_gfp	CCGGGATCCGATGGTTTGCAC CGATTC	Reverse primer for amplification of leuL attenuator region
thrA_fwd_gfp	CCGCTCGAGACTGCAACGGGC AATATG	Forward primer for amplification of thrL attenuator region
thrA_rev_gfp	CCGGGATCCTCGGCATCGCTG ATATTG	Reverse primer for amplification of thrL attenuator region