

Table S3. iJL1678-ME model modification (blocked reactions)

Iter.	Cat.	ID	Name	Formula	Subsystem	Comments (When the reaction is turned on)
1	bp2	EDD	6-phosphogluconate dehydratase	6pgc_c $\rightarrow$ 2ddg6p_c + h2o_c	Pentose Phosphate Pathway	Create a major effect of steep acetate overflow elevation in high growth. Comparing to the main glycolytic pathway, it is metabolically less efficient but proteomically more efficient.
	bp1	ICL	Isocitrate lyase	icit_c $\rightarrow$ glx_c + succ_c	Anaplerotic Reactions	Bypass for the main TCA cycle pathways from turning isocitrate to succinate, when ICL is turned on, Isocitrate dehydrogenase(ICDHr), 2-Oxoglutarate dehydrogenase(AKGDH) and Succinyl-CoA synthetase (ATP-forming,SUCOAS) would reduce. Ref. (1) and (2) shows that this reaction is off in higher growth. Ref. (3) shows that this reaction is converging to being off when the dynamic of respiration using enzyme kinetics is simulated.
2	bp1	ABTA	4-aminobutyrate transaminase	4abut_c + akg_c $\rightarrow$ glu_L_c + succal_c	Arginine and Proline Metabolism	Another backup pathway of succinate production, from 2-Oxoglutarate (akg). Respiration would be induced when it is on, since the flux through ETC(CYTBO3_4pp and ATPS4pp) would increase. As it requires the co-factor pyridoxal 5'-phosphate(2-) to get catalyzed(4), indicating that this reaction is regulated by the flux of other reactions(pyridoxal 5'-phosphate(2-) production, etc.).
3		GLYAT	Glycine C-acetyltransferase	accoa_c + gly_c $\rightarrow$ 2aobut_c + coa_c	Glycine and Serine Metabolism	A reaction that back up for the respiration. Reactions fluxes in TCA cycle would drop when this reaction is turned on. It also requires pyridoxal 5'-phosphate(2-) for the regulation.
4		NADTRHD	NAD transhydrogenase	nad_c + nadph_c $\rightarrow$ nadh_c + nadp_c	Oxidative Phosphorylation	A reaction that would make the transition between NAD and NADP metabolically more efficient. A notable effect when this reaction is turned on is that the reaction fluxes through ETC is elevated. Moreover, fluxes are yielded from the glycolytic pathway(EMP pathway) to the pentose phosphate pathway(PPP pathway, NADP synthesis). Ref(1) and (2) shows that the experimental flux through PPP is lower than the model's prediction, which indicates an extra regulation constraints upon NAD vs. NADP, EMP vs. PPP is missing.
5		ASPT	L-aspartase	asp_L_c $\rightarrow$ fum_c + nh4_c	Alanine and Aspartate Metabolism	Ref. (1) and (2) shows that this reaction is off in higher growth. Ref.
6		IDOND	L-idonate 5-dehydrogenase	5dglcn_c + h_c + nadh_c $\rightarrow$ idon_L_c + nad_c	Alternate Carbon Metabolism	Building up a futile cycle with the reaction ICOND2, with a combining effect of NAD/NADP switching through transhydrogenase. It requires zinc ion as cofactor (5).
7		FADRx2	FAD reductase	fad_c + h_c + nadph_c $\rightarrow$ fadh2_c + nadp_c	Cofactor and Prosthetic Group Biosynthesis	It requires FAD and FMN as cofactor.
8		DAAD	D-Amino acid dehydrogenase	ala_D_c + fad_c + h2o_c $\rightarrow$ fadh2_c + nh4_c +	Alanine and Aspartate Metabolism	Inducing the glutamate synthesis from 2-Oxoglutarate
9		FTHFD	Formyltetrahydrofolate deformylase	10fthf_c + h2o_c $\rightarrow$ for_c + h_c + thf_c	Folate Metabolism	This reaction induce the respiration fluxes by degrading the purine synthesis substrate 10-Formyltetrahydrofolate to forlate, the forlate is then pumped to periplasm for transporting electron. There's enzyme regulations (purU) upon this reaction, which is activated by the methionine which costs the most enzyme (6) to get synthesized, and at the mean time, this reaction is inhibited by the glucogenic amino acid glycine (7).
10		TRSARr	Tartronate semialdehyde reductase	2h3oppa_n_c + h_c + nadh_c $\rightarrow$ glyc_R_c + nad_c	Alternate Carbon Metabolism	It is the upstream of the reaction hydroxypyruvate reductase (NADPH), however, the corresponding gene (ghrA/ghrB) is more preferable in reducing glyoxylate than hydroxypyruvate (8).
11		ALATA_L2	Alanine transaminase	ala_L_c + pydx5p_c $\rightarrow$ pyam5p_c + pyr_c	Cofactor and Prosthetic Group Biosynthesis	The encoding genes, glyA and ItaE, has many other roles, where this reaction might be regulated by enzyme saturation.
12		FLDR22	Flavodoxin reductase (NADPH)	2.0 FLAVODOXIN2-MONOMER_mod_Oxidized	Flavodoxin reductase (NADPH)	The gene (fpr, fldA, fldB) requires FAD (9) or FMN (10) as cofactor. The effect of turning on this reaction is that it would reverse the direction of POR5 (Pyruvate synthase) from oxidizing pyruvate to reducing flavodoxin, where the gene (ydbK) requires [4Fe-4S] cluster as cofactor.
13		FLDR21	Flavodoxin reductase (NADPH) (For flavodoxin1 monomer)	2.0 FLAVODOXIN1-MONOMER_mod_Oxidized	Flavodoxin reductase (NADPH)	
14		PYAM5PO	Pyridoxamine 5'-phosphate oxidase	h2o_c + o2_c + pyam5p_c $\rightarrow$ h2o2_c + nh4_c +	Cofactor and Prosthetic Group Biosynthesis	The gene (pdxH) requires FMN as cofactor. The model recording its keff=40 (1/s), however some study (11) shows that its kcat is 0.3 (1/s)
15		VPAMTr	Valine-pyruvate aminotransferase	3mob_c + ala_L_c $\rightarrow$ pyr_c + val_L_c	Valine, Leucine, and Isoleucine Metabolism	
16		NADPHQR2	NADPH Quinone Reductase (Ubiquinone)	h_c + nadph_c + q8_c $\rightarrow$ nadp_c + q8h2_c	Oxidative Phosphorylation	Trading off NADH quinone reductase (NADH16pp), which has similar effect as NAD(P) transhydrogenase (NADTRHD), changing the NAD/NADP balance and make more carbon fluxes yielding to pentose phosphate pathway.
17		CTECOAI7	3-cis-2-trans-enoyl-CoA isomerase	hdcoa_c $\rightarrow$ hdd2coa_c	Membrane Lipid Metabolism	Acetyl-CoA metabolism
		AACPS31	Acyl-[acyl-carrier-protein] synthetase (n-C)	1.0 EG50003-MONOMER_mod_pan4p + atp_c	Cell Envelope Biosynthesis	Acetyl-CoA metabolism
		AACPS41	Acyl-[acyl-carrier-protein] synthetase (n-C)	1.0 EG50003-MONOMER_mod_pan4p + atp_c	Cell Envelope Biosynthesis	
		PUNP5	Purine-nucleoside phosphorylase (Inosine)	ins_c + pi_c $\rightarrow$ hxan_c + r1p_c	Nucleotide Salvage Pathway	Adenine metabolism
		R15BPk	Ribose-1,5-bisphosphokinase	atp_c + r15bp_c $\rightarrow$ adp_c + prpp_c	Alternate Carbon Metabolism	Upstream of histidine production
		FACOAIE161	Fatty-acid-CoA thioesterase (hexadecanoate)	h2o_c + hdcoa_c $\rightarrow$ coa_c + h_c + hdcea_c	Cell Envelope Biosynthesis	
		NTD11	5'-nucleotidase (IMP)	h2o_c + imp_c $\rightarrow$ ins_c + pi_c	Nucleotide Salvage Pathway	
		HXAND	Hypoxanthine dehydrogenase	h2o_c + hxan_c + nad_c $\rightarrow$ h_c + nadh_c + xan_c	Nucleotide Salvage Pathway	Adenine metabolism
		XPPT	Xanthine phosphoribosyltransferase	prpp_c + xan_c $\rightarrow$ ppi_c + xmp_c	Nucleotide Salvage Pathway	Guanine metabolism
		R1PK	Ribose 1-phosphokinase	atp_c + r1p_c $\rightarrow$ adp_c + h_c + r15bp_c	Alternate Carbon Metabolism	
18	bp2	EAR160x1	Enoyl-[acyl-carrier-protein] reductase (NA)	1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	Acetyl-CoA to Hexadecanoate (palmitic acid)
		EAR161x	Enoyl-[acyl-carrier-protein] reductase (NA)	1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	
		FACOAIE120	Fatty-acid-CoA thioesterase (dodecanoate)	ddcoa_c + h2o_c $\rightarrow$ coa_c + ddca_c + h_c	Cell Envelope Biosynthesis	
		FACOAIE140	Fatty-acid-CoA thioesterase (tetradecanoate)	h2o_c + tdcoa_c $\rightarrow$ coa_c + h_c + ttdca_c	Cell Envelope Biosynthesis	
		FACOAIE141	Fatty-acid-CoA thioesterase (tetradecanoate)	h2o_c + tdecoa_c $\rightarrow$ coa_c + h_c + ttdcea_c	Cell Envelope Biosynthesis	
		AACPS11	Acyl-[acyl-carrier-protein] synthetase (n-C)	1.0 EG50003-MONOMER_mod_pan4p + atp_c	Cell Envelope Biosynthesis	
		AACPS21	Acyl-[acyl-carrier-protein] synthetase (n-C)	1.0 EG50003-MONOMER_mod_pan4p + atp_c	Cell Envelope Biosynthesis	
		AACPS71	Acyl-[acyl-carrier-protein] synthetase (n-C)	1.0 EG50003-MONOMER_mod_pan4p + atp_c	Cell Envelope Biosynthesis	
		RNTR1c22	Ribonucleoside-triphosphate reductase (U)	2.0 FLAVODOXIN2-MONOMER + atp_c + 2.0 H	Nucleotide Salvage Pathway	
		RNTR2c22	Ribonucleoside-triphosphate reductase (U)	2.0 FLAVODOXIN2-MONOMER + 2.291649785	Nucleotide Salvage Pathway	
		RNTR3c22	Ribonucleoside-triphosphate reductase (U)	2.0 FLAVODOXIN2-MONOMER + ctp_c + 2.0 H	Nucleotide Salvage Pathway	
		RNTR4c22	Ribonucleoside-triphosphate reductase (U)	2.0 FLAVODOXIN2-MONOMER + 2.0 h_c + utp	Nucleotide Salvage Pathway	
		CTECOAI6	3-cis-2-trans-enoyl-CoA isomerase	tdecoa_c $\rightarrow$ td2coa_c	Membrane Lipid Metabolism	
20	bp2	FACOAIE80	Fatty-acid-CoA thioesterase (octanoate)	h2o_c + octcoa_c $\rightarrow$ coa_c + h_c + octa_c	Cell Envelope Biosynthesis	Acetyl-CoA metabolism
		FACOAIE100	Fatty-acid-CoA thioesterase (decanoate)	dcacoa_c + h2o_c $\rightarrow$ coa_c + dca_c + h_c	Cell Envelope Biosynthesis	
		AACPS81	Acyl-[acyl-carrier-protein] synthetase (n-C)	1.0 EG50003-MONOMER_mod_pan4p + atp_c	Cell Envelope Biosynthesis	
		AACPS91	Acyl-[acyl-carrier-protein] synthetase (n-C)	1.0 EG50003-MONOMER_mod_pan4p + atp_c	Cell Envelope Biosynthesis	
		EAR120x1	Enoyl-[acyl-carrier-protein] reductase (NA)	1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	
		EAR121x1	Enoyl-[acyl-carrier-protein] reductase (NA)	1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	
		EAR140x1	Enoyl-[acyl-carrier-protein] reductase (NA)	1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	
		EAR141x1	Enoyl-[acyl-carrier-protein] reductase (NA)	1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	
		RNTR2c21	Ribonucleoside-triphosphate reductase (U)	2.0 FLAVODOXIN1-MONOMER + gtp_c + 2.0 H	Nucleotide Salvage Pathway	
		RNTR3c21	Ribonucleoside-triphosphate reductase (U)	2.0 FLAVODOXIN1-MONOMER + ctp_c + 2.0 H	Nucleotide Salvage Pathway	
		RNTR4c21	Ribonucleoside-triphosphate reductase (U)	2.0 FLAVODOXIN1-MONOMER + 2.0 h_c + utp	Nucleotide Salvage Pathway	
		POR51	Pyruvate synthase	2.0 FLAVODOXIN1-MONOMER + accoa_c + cd	Pyruvate Metabolism	
		21	bp2	EAR40x1	Enoyl-[acyl-carrier-protein] reductase (NA)	
EAR60x1	Enoyl-[acyl-carrier-protein] reductase (NA)			1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	Acetyl-CoA metabolism
EAR80x1	Enoyl-[acyl-carrier-protein] reductase (NA)			1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	Acetyl-CoA metabolism
EAR100x1	Enoyl-[acyl-carrier-protein] reductase (NA)			1.0 EG50003-MONOMER_mod_pan4p_mod_t	Cell Envelope Biosynthesis	Acetyl-CoA metabolism

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Table S4. Essential exchanges		
Num.	ID	Exchanging component
1	EX_5mtr_e	5-Methylthio-D-ribose
2	EX_RNase_m16	Ribonuclease M16
3	EX_RNase_m23	Ribonuclease M23
4	EX_RNase_m5	Ribonuclease M5
5	EX_ac_e	Acetate
6	EX_co2_e	CO <sub>2</sub>
7	EX_cobalt2_e	Co <sup>2+</sup>
8	EX_cu2_e	Cu <sup>2+</sup>
9	EX_fe2_e	Fe <sup>2+</sup>
10	EX_glc_D_e	D-Glucose
11	EX_h2o_e	H <sub>2</sub> O
12	EX_h_e	H <sup>+</sup>
13	EX_k_e	potassium
14	EX_meoh_e	Methanol
15	EX_mg2_e	magnesium
16	EX_mn2_e	Mn <sup>2+</sup>
17	EX_mobd_e	Molybdate
18	EX_nh4_e	Ammonium
19	EX_o2_e	O <sub>2</sub>
20	EX_pi_e	Phosphate
21	EX_slnt_e	selenite
22	EX_so4_e	Sulfate
23	EX_tl_c	thallium ion
24	EX_zn2_e	Zinc

Table S5. Solution space variation (Below)

Iter.	ID	Name	Reaction	Subsystem	Comments (effects when the reaction is turned on)
1	GLCt2pp	D-glucose transport in via proton symport (periplasm)	$glc\_D\_p + h\_p \rightleftharpoons glc\_D\_c + h\_c$	Transport, Inner Membrane	The energetic efficiency of respiration gets reduced. Succinyl-CoA synthetase (ADP-forming) gets turned off. More protons get produced in the cytosol by CO <sub>2</sub> through HCO <sub>3</sub> equilibration reaction. Flux through pentose phosphate pathway gets induced where yielding the glycolytic flux. Less ATP is synthesized through ATP synthase.
2	GLYCLTDy	Glycolate dehydrogenase (NADP)	$glx\_c + h\_c + nadph\_c \rightarrow glyclt\_c + nadp\_c$	Glyoxylate Metabolism	Absorbing protons into the metabolites, causing an energy waste. At the meantime, instead of pumping protons, quinone are reduced through Glycolate oxidase, wasting the energy source within the electric transport chain.
3	G3PD5	Glycerol-3-phosphate dehydrogenase (ubiquinone-8)	$glyc3p\_c + q8\_c \rightarrow dhap\_c + q8h2\_c$	Oxidative Phosphorylation	A energetic wasteful alternative of the ETC reactions.
4	NADPHQR3	NADPH Quinone Reductase (Menaquinone-8)	$h\_c + mqn8\_c + nadph\_c \rightarrow mql8\_c + nadp\_c$	Oxidative Phosphorylation	Yielding the glycolytic flux to pentose phosphate pathway. Wasting one proton. Fumarate reductase (FRD2) gets activated, yielding the flux from acetate production to TCA cycle.
5	NADPHQR4	NADPH Quinone Reductase (2-Demethylmenaquinone-8)	$2dmmq8\_c + h\_c + nadph\_c \rightarrow 2dmmql8\_c + nadp\_c$	Oxidative Phosphorylation	Yielding the glycolytic flux to pentose phosphate pathway. Wasting one proton. Fumarate reductase (FRD3) gets activated, yielding the flux from acetate production to TCA cycle.
6	SERD_L	L-serine deaminase	$ser\_L\_c \rightarrow nh4\_c + pyr\_c$	Glycine and Serine Metabolism	Both reactions are degrading amino acids to pyruvate. The flux through pyruvate kinase (PYK) is eliminated, where the PYK ATP production is yielded to ATP synthase. More CO <sub>2</sub> is also secreted, showing that the respiration got level up, delaying the onset of acetate production.
7	TRPAS2	Tryptophanase (L-tryptophan)	$h2o\_c + trp\_L\_c \rightleftharpoons indole\_c + nh4\_c + pyr\_c$	Tyrosine, Tryptophan, and Phenylalanine Metabolism	
8	G3PD6	Glycerol-3-phosphate dehydrogenase (menaquinone-8)	$glyc3p\_c + mqn8\_c \rightarrow dhap\_c + mql8\_c$	Oxidative Phosphorylation	A energetic wasteful alternative of the ETC reactions. Fumarate reductase (FRD2) gets activated, yielding the flux from acetate production to TCA cycle.
9	G3PD7	Glycerol-3-phosphate dehydrogenase (demethylmenaquinone-8)	$2dmmq8\_c + glyc3p\_c \rightarrow 2dmmql8\_c + dhap\_c$	Oxidative Phosphorylation	A energetic wasteful alternative of the ETC reactions. Fumarate reductase (FRD3) gets activated, yielding the flux from acetate production to TCA cycle.
10	LALDO3	L-Lactaldehyde:NADP+ 1-oxidoreductase	$h\_c + mthgl\_c + nadph\_c \rightarrow lald\_L\_c + nadp\_c$	Methylglyoxal Metabolism	Wasting a proton as an alternative of the ETC reactions. The flux through pyruvate kinase (PYK) is eliminated. CO <sub>2</sub> secretion is leveled up.
11	LSERDhr	L-serine dehydrogenase	$nadp\_c + ser\_L\_c \rightleftharpoons 2amsa\_c + h\_c + nadph\_c$	Glycine and Serine Metabolism	The flux through pyruvate kinase (PYK) is eliminated. More CO <sub>2</sub> is secreted.

Table S6. Solution space variation (Above)						
Iter.	ID	Name	Reaction	Subsystem	Comments (effects when the reaction is turned on)	Secondary Comments
1	THD2pp	NAD(P) transhydrogenase (periplasm)	$2.0 h_p + nadh_c + nadp_c \rightarrow 2.0 h_c + nad_c + nadph_c$	Oxidative Phosphorylation	A proton pump gradient against the ATP synthase (ATPS4rpp). Flux through pentose phosphate pathway is yielding to main glycolytic pathway.	
2	NADH5	NADH dehydrogenase (ubiquinone-8 )	$h_c + nadh_c + q8_c \rightarrow nad_c + q8h2_c$	Oxidative Phosphorylation	Trading off NADH dehydrogenase (NADH16pp, which pumps 3 protons to the periplasm), . Via NADH5, no proton get pumped to the periplasm.	
3	ALDD2y	Aldehyde dehydrogenase (acetaldehyde, NADP)	$acald_c + h2o_c + nadp_c \rightarrow ac_c + 2.0 h_c + nadph_c$	Alternate Carbon Metabolism	With the inverse acetaldehyde dehydrogenase (acetylating, ACALD, $accoa_c + h_c + nadh_c \rightarrow acald_c + coa_c + nad_c$ ), a futile cycle for acetaldehyde is created, with extra acetate production.	
4	POX	Pyruvate oxidase	$h2o_c + pyr_c + q8_c \rightarrow ac_c + co2_c + q8h2_c$	Oxidative Phosphorylation	An acetate production reaction that alternates the proton pumping quinone reductase in electric transport chain. The flux through acetate kinase (ACKr) gets reduced.	
5	ME2	Malic enzyme (NADP)	$mal\_L_c + nadp_c \rightarrow co2_c + nadph_c + pyr_c$	Anaplerotic Reactions	Trading off malate dehydrogenase (MDH), the second last step of the TCA cycle, turning malate oxaloacetate, then to citrate. ME2 turns malate to pyruvate, emitting CO2, and feeding more pyruvate for acetate production.	
6	LDH_D2	D-lactate dehydrogenase	$lac\_D_c + q8_c \rightarrow pyr_c + q8h2_c$	Oxidative Phosphorylation	Alternative for the proton pumping quinone reductase. Flux through pentose phosphate pathway and cytochrome oxidase get increased. ATP synthase flux also increases. Main glycolytic pathway fluxes are reduced.	
7	GLYCLTDx	Glycolate dehydrogenase (NAD)	$glx_c + h_c + nadh_c \rightarrow glyclt_c + nad_c$	Glyoxylate Metabolism	Pentose phosphate pathway fluxes get induced, yielded from the main glycolytic pathway.	
8	NADH10	NADH dehydrogenase (menaquinone-8 & 0 protons)	$h_c + mqn8_c + nadh_c \rightarrow mql8_c + nad_c$	Oxidative Phosphorylation	Introducing a futile cycle between fumarate and succinate in the TCA cycle, via fumarate reductase (FRD2) and succinate dehydrogenase (SUCDi), and consequently trading off NADH16pp as a poor proton pump alternatives.	
9	MDH3	Malate dehydrogenase (menaquinone 8 as acceptor)	$mal\_L_c + mqn8_c \rightarrow mql8_c + oaa_c$	Citric Acid Cycle		
10	MDH2	Malate dehydrogenase (ubiquinone 8 as acceptor)	$mal\_L_c + q8_c \rightarrow oaa_c + q8h2_c$	Citric Acid Cycle		
11	CU2R	NADH:quinone oxidoreductase II	$cu2_c + nadh_c \rightarrow cu_c + h_c + nad_c$	Oxidative Phosphorylation		
12	LDH_D	D-lactate dehydrogenase	$lac\_D_c + nad_c \rightleftharpoons h_c + nadh_c + pyr_c$	Pyruvate Metabolism		
13	MALt2_3pp	Malate transport via proton symport (3 H) (periplasm)	$3.0 h_p + mal\_L_p \rightarrow 3.0 h_c + mal\_L_c$	Transport, Inner Membrane	Causing the pathway switch that malate dehydrogenase (MDH, $mal\_L_c + nad_c \rightleftharpoons h_c + nadh_c + oaa_c$ ) shifting to malic enzyme (ME1, $mal\_L_c + nad_c \rightleftharpoons co2_c + nadh_c + pyr_c$ ), and pyruvate kinase (PYK, $adp_c + h_c + pep_c \rightleftharpoons atp_c + pyr_c$ ) shifting to phosphoenolpyruvate carboxylase (PPC, $co2_c + h2o_c + pep_c \rightleftharpoons h_c + oaa_c + pi_c$ ), causing the overall effect of wasting 1 atp molecule.	Introducing TCA intermediate metabolites, which reduces the TCA flux, yielding to the backup pathways
14	SUCct3pp	Succinate transport out via proton antiport (periplasm)	$h_p + succ_c \rightarrow h_c + succ_p$	Transport, Inner Membrane		
15	FE2t3pp	Iron (Fe+2) transport out via proton antiport (periplasm)	$fe2_c + h_p \rightarrow fe2_p + h_c$	Inorganic Ion Transport and Metabolism		
16	ALDD2x	Aldehyde dehydrogenase (acetaldehyde, NAD)	$acald_c + h2o_c + nad_c \rightarrow ac_c + 2.0 h_c + nadh_c$	Alternate Carbon Metabolism		
17	MALt2_2pp	Malate transport via proton symport (2 H) (periplasm)	$2.0 h_p + mal\_L_p \rightarrow 2.0 h_c + mal\_L_c$	Transport, Inner Membrane		
18	GART	GAR transformylase-T	$atp_c + for_c + gar_c \rightarrow adp_c + fgam_c + h_c + pi_c$	Purine and Pyrimidine Biosynthesis		
19	FTHFLi	Formate-tetrahydrofolate ligase	$atp_c + for_c + thf_c \rightarrow 10thf_c + adp_c + pi_c$	Folate Metabolism		
20	MG2tpp	Magnesium (+2) transport in via diffusion	$mg2_p \rightarrow mg2_c$	Inorganic Ion Transport and Metabolism	Causing the pathway switch that malate dehydrogenase (MDH, $mal\_L_c + nad_c \rightleftharpoons h_c + nadh_c + oaa_c$ ) shifting to malic enzyme (ME1, $mal\_L_c + nad_c \rightleftharpoons co2_c + nadh_c + pyr_c$ ), and pyruvate kinase (PYK, $adp_c + h_c + pep_c \rightleftharpoons atp_c + pyr_c$ ) shifting to phosphoenolpyruvate carboxylase (PPC, $co2_c + h2o_c + pep_c \rightleftharpoons h_c + oaa_c + pi_c$ ), causing the overall effect of wasting 1 atp molecule.	Lower energetic efficiencies, comparing to the Magnesium (Mg+2) transport in/out via proton antiport (periplasm) (MG2t3_2pp, $2.0 h_c + mg2_p \rightarrow mg2_c + 2.0 h_p$ ).
21	MG2uabcpp	Magnesium (Mg+2) ABC transporter (ubtake, periplasm)	$atp_c + h2o_c + mg2_p \rightarrow adp_c + h_c + mg2_c + pi_c$	Inorganic Ion Transport and Metabolism		
22	FAC0AE180	Fatty-acid-CoA thioesterase (octadecanoate)	$h2o_c + stcoa_c \rightarrow coa_c + h_c + ocdca_c$	Cell Envelope Biosynthesis		
23	AACPS51	Acyl-[acyl-carrier-protein] synthetase (n-C18:1)	$ACP_c + atp_c + ocdcea_c \rightarrow amp_c + octeACP_c + ppi_c$	Cell Envelope Biosynthesis		
24	ADPT	Adenine phosphoribosyltransferase	$ade_c + prpp_c \rightarrow amp_c + ppi_c$	Nucleotide Salvage Pathway		

Table S7. ALE phenotypes measurements

Strain	Growth Rate (1/h)				Glucose uptake rate (mmol/gDW/h)				Acetate production rate (mmol/gDW/h)			
	Rate rep. 1	Rate rep 2	avg rep 1 and 2	std rep 1 and 2	Rate rep. 1	Rate rep 2	avg rep 1 and 2	std rep 1 and 2	Rate rep. 1	Rate rep 2	avg rep 1 and 2	std rep 1 and 2
3	1.106	1.015	<b>1.016</b>	<b>0.001</b>	12.007	12.55	<b>12.278</b>	<b>0.384</b>	7.76	7.708	<b>7.734</b>	<b>0.037</b>
4	0.943	0.953	<b>0.948</b>	<b>0.008</b>	11.677	11.639	<b>11.658</b>	<b>0.027</b>	7.485	7.522	<b>7.504</b>	<b>0.026</b>
6	1.008	1.021	<b>1.014</b>	<b>0.009</b>	12.91	12.629	<b>12.77</b>	<b>0.199</b>	10.329	9.763	<b>10.046</b>	<b>0.4</b>
7*	1.085	1.067	<b>1.076</b>	<b>0.013</b>	12.723	12.948	<b>12.835</b>	<b>0.159</b>	6.062	6.445	<b>6.254</b>	<b>0.271</b>
7A*	1.075	1.01	<b>1.042</b>	<b>0.046</b>	11.268	10.407	<b>10.837</b>	<b>0.609</b>	5.432	4.637	<b>5.035</b>	<b>0.562</b>
7B*	0.989	0.946	<b>0.967</b>	<b>0.031</b>	10.108	10.135	<b>10.122</b>	<b>0.019</b>	3.786	3.905	<b>3.845</b>	<b>0.084</b>
8	1.078	1.094	<b>1.086</b>	<b>0.012</b>	13.252	13.355	<b>13.304</b>	<b>0.073</b>	8.224	8.715	<b>8.469</b>	<b>0.348</b>
9	0.971	1.032	<b>1.002</b>	<b>0.043</b>	11.653	13.446	<b>12.549</b>	<b>1.268</b>	7.925	8.977	<b>8.451</b>	<b>0.744</b>
10	1.17	1.035	<b>1.102</b>	<b>0.095</b>	14.847	13.623	<b>14.235</b>	<b>0.866</b>	11.841	10.895	<b>11.368</b>	<b>0.669</b>

Table S8. MG1655 WT phenotypes measurements.										
Strain No.	Growth Rate (1/h)	Growth Rate CI	Glucose UR (mmol/gDW/h)	Glucose UR CI	Acetate PR (mmol/gDW/h)	Acetate PR CI	Biomass Yield (gDW/gGlc)	Biomass Yield CI		Data taken from:
0	0.69	0.02	8.59	1.42	3.91	1.14	0.44	0.07		Appl Environ Microbiol. 2015 Jan 1;81(1):17-30. doi: 10.1128/AEM.02246-14
Cite from: LaCroix RA, et al. (2015) Use of adaptive laboratory evolution to discover key mutations enabling rapid growth of Escherichia coli K-12 MG1655 on glucose minimal medium. Appl Environ Microbiol 81(1):17–30.										

Table S9. NCM3722 uptake titration phenotypes

Growth (1/hr)	Glucose uptake (mM/OD600/hr)	Acetate excretion rate (mmol/OD600/hr)
0.88	5.58	1.88
0.81	4.94	0.7
0.75	4.31	0
0.71	3.9	0
0.68	3.94	0
0.58	3.25	0
0.97	5.4	2.52
0.94	4.97	2.37
0.91	5.48	2.38
0.88	5.11	1.94
0.87	5.15	1.5
0.81	4.69	1.16
0.78	4.46	0.03
0.74	4.21	0

**Growth yield (gDW/g\_glucose) = Growth (1/hr) / Glucose uptake (mM/OD600/hr) / 180.1559 (g/mol) / 2 (mM/OD600/(mmol/gDW)) \* 1000 (mmol/mol)**

Cite from: Basan M, et al. (2015) Overflow metabolism in Escherichia coli results from efficient proteome allocation. Nature 528(7580):99–104.

Table S10. MG1655 chemostat measurements		
Growth (1/hr)	Growth Yield (gDW/g_glucose)	Acetate concentration ((g_acetate/L) / (g_glucose/L) )
0.047216035634744	0.229224178287206	0
0.0436525612472161	0.271268069169989	0
0.0579064587973274	0.282710340033762	0
0.0846325167037862	0.28585249599251	0
0.0962138084632517	0.318955073553403	0
0.114031180400891	0.323385300668152	0
0.0472160356347439	0.337504433064276	0
0.110467706013363	0.36097059282482	0
0.121158129175947	0.353310257756089	0
0.205790645879733	0.374831543557516	0
0.187973273942094	0.371675201793086	0
0.212026726057907	0.38182798292029	0
0.285968819599109	0.375977756656689	0
0.315367483296214	0.38102648489921	0
0.301113585746102	0.383596952888939	0
0.405345211581292	0.401902316542068	0.791068580542264
0.40890868596882	0.391705559417247	0
0.398218262806236	0.396181181110181	0
0.512249443207127	0.418929539103173	0.284822606499937
0.547884187082405	0.418872795880442	1.15051139829487
0.587082405345212	0.414988722284482	1.65944136297221
0.692204899777283	0.375330883917552	3.0208247627424
washout=0.7 (1/hr)		
Acetate excretion rate= Acetate concentration / washout		
Glucose uptake rate= Growth / Growth Yield / 180.1559 * 1000		
Cite from: Nanchen A, Schicker A, Sauer U (2006) Nonlinear dependency of intracellular fluxes on growth rate in miniaturized continuous cultures of Escherichia coli		