Multi-omics analysis unravels a segregated metabolic flux network that tunes co-utilization of sugar and aromatic carbons in *Pseudomonas putida*

Matthew A. Kukurugya[‡,1], Caroll M. Mendonca [‡,1], Mina Solhtalab[‡], Rebecca A. Wilkes[‡], Theodore W. Thannhauser[§], and Ludmilla Aristilde[‡,2]

From the [‡]Department of Biological and Environmental Engineering, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853, USA. [§]United States Department of Agriculture—Agricultural Research Service, Ithaca, NY 14853, USA.

[1]Co-first authors

[2]To whom correspondence should be addressed: 214 Riley-Robb Hall, Cornell University, Ithaca, NY 14850; Tel. (607) 255-6845; Fax. (607) 255-4449; ludmilla@cornell.edu

Running title: *Segregated metabolism for mixed-substrate usage in P. putida*

Keywords: *Pseudomonas*, bacterial metabolism, metabolomics, proteomics, glucose, benzoate, mixed carbon metabolism

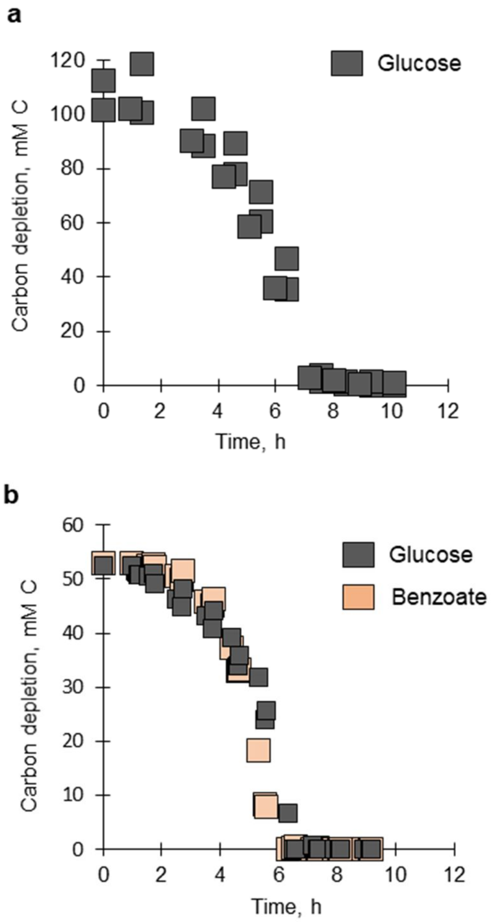
SUPPLEMENTARY INFORMATION

Appendix G

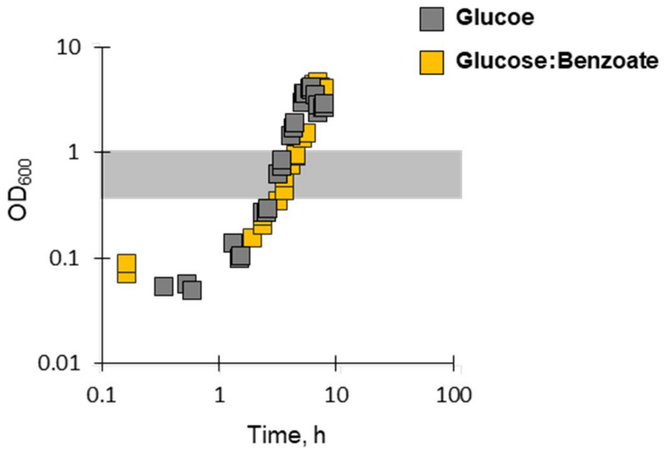
Supplementary Figure 5

Supplementary Figure 6

Appendix A

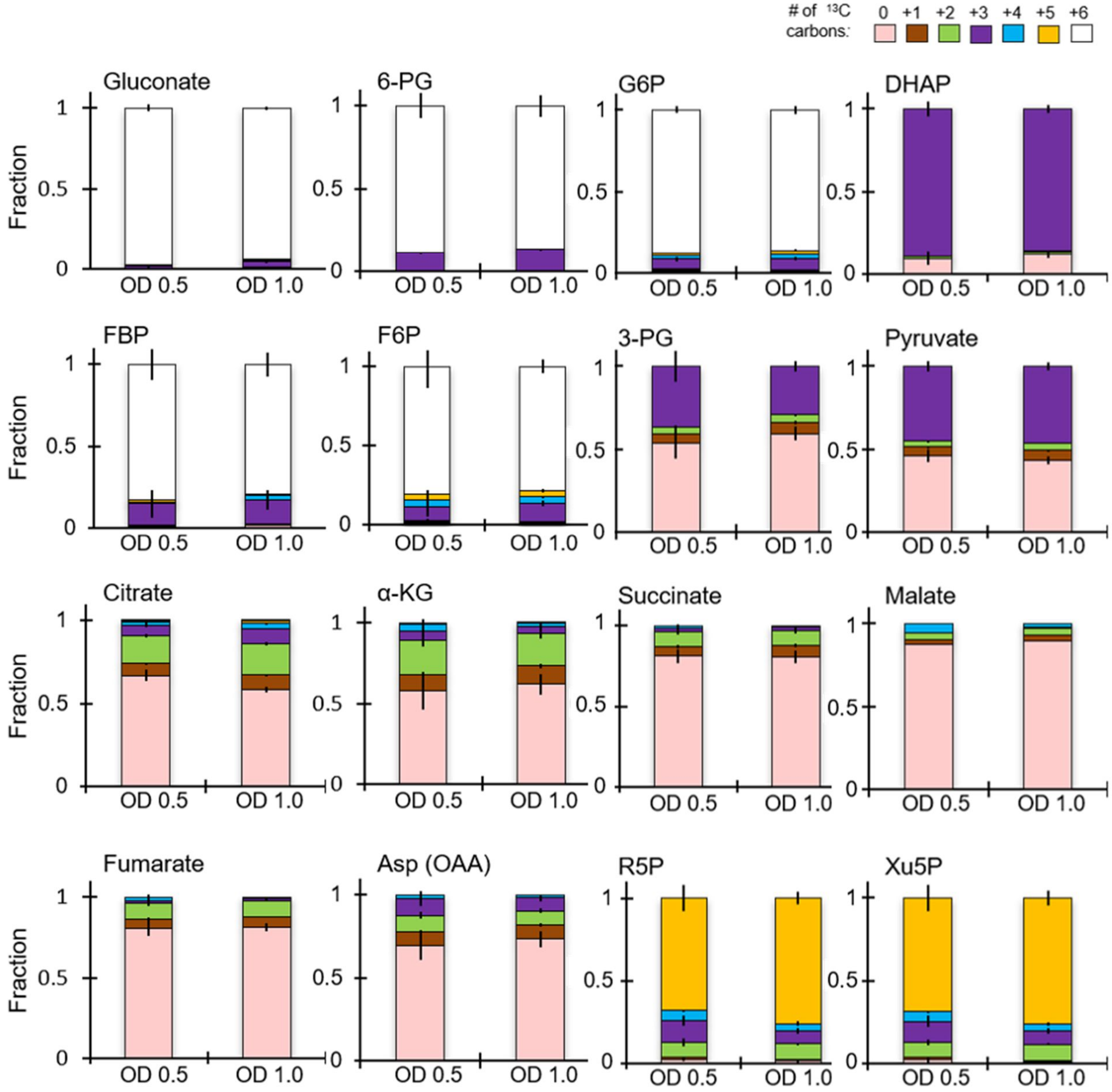


Supplementary Figure 1. Kinetics of extracellular depletion by *P. putida* KT2440 grown on (A) glucose alone or (B) both glucose and benzoate.



Supplementary Figure 2. Growth curves of *P. putida* KT2440 cells on glucose alone (dark gray) or both glucose and benzoate (light orange). The light gray box indicates the OD values, which were chosen to obtain exponentially-growing cells for retrieve metabolomics data (0.5 and 1.1 ODs) and proteomics data (0.5-0.7 ODs).

Appendix B



Supplementary Figure 3. Labeling profile of central carbon metabolites during growth on [U-$^{13}C_6$]-glucose with unlabeled benzoate. Error bars represent standard deviation obtained from three biological replicates. Refer to Fig. 1 (main text) for metabolite abbreviations.

Appendix C

Supplementary Table 1. List of the transporters, enzymes and isozymes corresponding to gene annotations shown in Fig. 1. The information for the network was compiled from the databases BioCyc, MetaCyc, UniProt and several studies available in the literature (del Castillo et al., 2007; Sudarsan et al., 2014; Nikel et al., 2016). In the instances in which no gene name has been assigned, the PP number is given for each open reading frame.

Open reading frame (ORF) no.	Gene annotation	Accession	Description [NCBI]
PP_3168	benF	NP_745312.1	porin-like protein
PP_3165	benK	NP_745309.1	benzoate MFS transporter
PP_3163	benC	NP_745307.1	benzoate 1,2-dioxygenase electron transfer component
PP_3162	benB	NP_745306.1	benzoate 1,2-dioxygenase subunit beta
PP_3161	benA	NP_745305.1	benzoate 1,2-dioxygenase subunit alpha
PP_3164	benD	NP_745308.1	1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate dehydrogenase
PP_3713	catA	NP_745846.1	catechol 1,2-dioxygenase
PP_3715	catB	NP_745848.1	muconate cycloisomerase 1
PP_3714	catC	NP_745847.1	muconolactone delta-isomerase
PP_1380	pcaD	NP_743539.1	3-oxoadipate enol-lactonase 2
PP_3952	pcaJ	NP_746082.1	3-oxoadipate CoA-transferase subunit A
PP_3951	pcaI	NP_746081.1	3-oxoadipate CoA-transferase subunit A
PP_1377	pcaF	NP_743536.1	beta-ketoadipyl-CoA thiolase
PP_1505	ppc	NP_743662.1	phosphoenolpyruvate carboxylase
PP_5346	OadA	NP_747447.1	pyruvate carboxylase subunit B
PP_5347	AccC-2	NP_747448.1	pyruvate carboxylase subunit A
PP_5085	maeB	NP_747186.1	malic enzyme B
PP_2925	mqo3	NP_745069.1	malate:quinone oxidoreductase
PP_1251	mqo2	NP_742912.1	malate:quinone oxidoreductase
PP_0751	mqo1	NP_743411.3	malate:quinone oxidoreductase

PP_0654	mdh	NP_742815.2	malate dehydrogenase
PP_1755	fumC-2	NP_743911.1	class 2 fumarate hydratase
PP_0944	fumC	NP_743105.1	class 2 fumarate hydratase
PP_4193	sdhC	NP_746310.1	succinate dehydrogenase membrane b-556 subunit
PP_4190	sdhB	NP_746307.1	succinate dehydrogenase iron-sulfur subunit
PP_4191	sdhA	NP_746308.1	succinate dehydrogenase flavoprotein subunit
PP_4185	sucD	NP_746302.1	succinyl-CoA synthetase subunit alpha
PP_4186	sucC	NP_746303.1	bifunctional succinyl-CoA synthetase subunit beta/glutaryl-CoA synthetase subunit beta
PP_5366	lpd	NP_747467.1	dihydrolipoyl dehydrogenase
PP_4404	lpdV	NP_746518.1	branched-chain alpha-keto acid dehydrogenase complex dihydrolipoyl dehydrogenase
PP_4187	lpdG	NP_746304.1	dihydrolipoyl dehydrogenase
PP_4188	sucB	NP_746305.1	2-oxoglutarate dehydrogenase dihydrolipoyltranssuccinylase subunit
PP_4189	sucA	NP_746306.1	2-oxoglutarate decarboxylase E1 component
PP_4011	icd	NP_746141.1	NADP(+)-specific isocitrate dehydrogenase
PP_4012	PP4012	NP_746142.1	isocitrate dehydrogenase
PP_2112	acnA	NP_744261.1	aconitate hydratase
PP_2339	acnB	NP_744488.1	bifunctional aconitate hydratase 2/2-methylisocitrate dehydratase
PP_4194	gltA	NP_746311.1	citrate synthase
PP_0356	glcB	NP_742523.1	malate synthase G
PP_4116	aceA	NP_746235.1	isocitrate lyase
PP_4965	tktA	NP_747068.1	transketolase A
PP_2168	tal	NP_744317.1	transaldolase
PP_0415	rpe	NP_742581.2	ribulose-5-phosphate 3-epimerase
PP_5150	rpiA	NP_747251.1	ribose-5-phosphate isomerase A
PP_4043	gnd	NP_746169.1	6-phosphogluconate dehydrogenase
PP_4701	pgi2	NP_746810.1	glucose 6-phosphate isomerase

PP_1808	pgi1	NP_743963.1	glucose 6-phosphate isomerase
PP_5040	fbp	NP_747141.1	class 1 fructose-1,6-bisphosphatase
PP_4960	fda	NP_747063.1	fructose-bisphosphate aldolase
PP_4715	tpiA	NP_746823.1	triose phosphate isomerase
PP_0338	aceF	NP_742505.1	AceF-S-acetyldihydrolipoate
PP_0399	aceE	NP_742506.1	pyruvate dehydrogenase E1 component
PP_4301	pykF	NP_746417.1	pyruvate kinase
PP_1362	pykA	NP_743521.1	pyruvate kinase II
PP_3082	ppsA	NP_744232.1	phosphoenolpyruvate synthetase
PP_1612	eno	NP_743769.1	enolase
PP_5056	pgm	NP_747157.1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase
PP_4963	pgk	NP_747066.1	phosphoglycerate kinase
PP_3443	PP3443	NP_745581.1	glyceraldehyde-3-phosphate dehydrogenase
PP_2149	gap-2	NP_744298.1	glyceraldehyde-3-phosphate dehydrogenase
PP_1024	eda	NP_743185.1	KHG/KDPG aldolase
PP_1010	edd	NP_743171.1	phosphogluconate dehydratase
PP_1022	zwfA	NP_743183.1	glucose 6-phosphate-1-dehydrogenase
PP_5351	Zwf	NP_747452.1	glucose 6-phosphate 1-dehydrogenase
PP_4042	zwfB	NP_746168.1	glucose 6-phosphate 1-dehydrogenase
PP_3416	gnuk	NP_745554.1	D-gluconate kinase
PP_1011	glk	NP_743172.1	glucokinase
PP_3376	kguD	NP_745516.1	phosphonate dehydrogenase
PP_3378	kguK	NP_745518.1	2-ketogluconokinase
PP_3377	kguT	NP_745517.1	2-ketogluconate transporter, putative
PP_3383	PP3383	NP_745523.1	gluconate 2-dehydrogenase flavoprotein subunit
PP_3384	PP3384	NP_745524.1	gluconate 2-dehydrogenase gamma subunit
PP_3382	PP3382	NP_745522.1	gluconate 2-dehydrogenase cytochrome c subunit

PP_3417	gntP	NP_745555.1	D-gluconate transporter
PP_1444	gcd	NP_743602.1	quinoprotein glucose dehydrogenase
PP_1018	gtsD	NP_743179.1	mannose/glucose ABC transporter ATP binding protein
PP_1017	gtsC	NP_743178.1	mannose/glucose ABC transporter permease
PP_1016	gtsB	NP_743177.1	mannose/glucose ABC transporter permease
PP_1015	gtsA	NP_743176.1	mannose/glucose ABC transporter substrate-binding protein
PP_1019	oprB-1	NP_743180.1	carbohydrate-selective porin

Appendix D

Supplementary Table 2. Intracellular metabolic rates determined from quantitative flux modeling of the metabolism of [1,5,6-$^{13}C_3$]-glucose alone or with unlabeled benzoate by *P. putida* KT2440 using the 13CFLUX2 software. These metabolic fluxes are illustrated in Fig. 4. The grey boxes indicate the absence of that reaction. Refer to the legends of Fig (main text) 1 for the metabolite abbreviations.

Reactions	**Glucose**	**Glucose:Benzoate**
	mmol gCDW^{-1} h^{-1}	
Gluc_{ext} → Gluc_{peri}	8.09 ± 0.01	3.26 ± 0.14
Gluc_{peri} → G6P	0.92 ± 0.31	0.60 ± 0.47
Gluc_{peri} → Glucn_{peri}	7.17 ± 0.31	2.66 ± 0.34
Glucn_{peri} → 6-PG	5.06 ± 0.17	2.07 ± 0.31
Glucn_{peri} → 2KGlucn_{peri}	0.90 ± 0.14	0.46 ± 0.03
2KGlucn_{peri} → 6-PG	0.90 ± 0.14	0.46 ± 0.03
G6P → 6-PG	1.30 ± 0.33	1.15 ± 1.02
6-PG → Pyruvate + GAP	7.16 ± 0.02	3.24 ± 0.48
GAP → 3PG	5.38 ± 0.03	0.52 ± 0.17
3PG → PEP	4.30 ± 0.03	-0.41 ± 0.10
PEP → Pyruvate	4.85 ± 0.03	-0.22 ± 0.07
DHAP → GAP	0.79 ± 0.02	1.19 ± 0.36
FBP → DHAP + GAP	0.71 ± 0.15	1.11 ± 0.26
FBP → F6P	0.71 ± 0.02	1.11 ± 0.37
F6P → G6P	0.49 ± 0.02	0.66 ± 0.54
6-PG → Ru5P + CO_2	0.10 ± 0.01	0.31 ± 0.20
Ru5P → Xu5P	-0.22 ± 0.01	-0.57± 0.17
Ru5P → R5P	0.32 ± 0.01	0.88 ± 0.03
Xu5P + R5P → GAP + S7P	0.05 ± 0.01	-0.10 ± 0.41
S7P + GAP → E4P + F6P	0.05 ± 0.01	-0.10 ± 0.41
F6P + GAP → E4P + Xu5P	0.27 ± 0.02	0.47 ± 0.08
Pyruvate → Acetyl-CoA + CO_2	8.41 ± 0.04	3.52 ± 0.97
Pyruvate + CO_2 → OAA	6.97 ± 0.05	-0.03 ± 0.12
OAA → PEP + CO_2	1.24 ± 0.01	0.92 ± 0.09
OAA + Acetyl-CoA → Citrate	8.41 ± 0.04	6.11 ± 1.28
Citrate → α-KG	8.41 ± 0.04	4.41 ± 3.39
α-KG → Succinate + CO_2	7.80 ± 0.05	3.31 ± 3.48
Succinate → Fumarate	7.78 ± 0.04	9.84 ± 1.07
Fumarate → Malate	7.86 ± 0.04	9.54 ± 1.40
Malate → OAA	3.63 ± 0.03	8.75 ± 0.83
Malate → Pyruvate + CO_2	4.24 ± 0.09	2.77 ± 0.96
OAA → Fumarate	0.08 ± 0.01	0.05 ± 0.03
Benz → Benz_{peri}		4.57 ± 0.14
Benz → Acetyl-CoA + Succinate		4.57 ± 0.14

Citrate → Glyoxylate + Succinate	0.00 ± 0.00	3.46 ± 0.01
Glyoxylate + Acetyl-CoA → Malate	0.00 ± 0.00	3.47 ± 0.01

Supplementary Table 3. Biomass and excretion efflux rates of metabolites determined from quantitative flux modeling of the metabolism of [1,5,6-$^{13}C_3$]-glucose alone or with unlabeled benzoate by *P. putida* KT2440 using the 13CFLUX2 software. These metabolic fluxes are illustrated in Fig. 4. Refer to the legends of Fig 1 (main text) for the metabolite abbreviations.

Reactions	**Glucose**	**Glucose:Benzoate**
	mmol gCDW^{-1} h^{-1}	
DHAP → Biomass	0.082 ± 0.0003	0.081 ± 0.009
E4P → Biomass	0.321 ± 0.0004	0.379 ± 0.012
G6P → Biomass	0.104 ± 0.001	0.106 ± 0.012
PEP → Biomass	0.680 ± 0.001	0.720 ± 0.064
Pyruvate → Biomass	0.873 ± 0.021	2.298 ± 0.437
R5P → Biomass	0.275 ± 0.001	0.980 ± 0.061
3PG → Biomass	1.083 ± 0.0004	0.933 ± 0.267
OAA → Biomass	0.869 ± 0.017	1.641 ± 0.210
α-KG → Biomass	0.605 ± 0.008	0.814 ± 0.092
Glucn$_{in}$ → Glucn$_{ext}$	1.209 ± 0.002	0.127 ± 0.008
2-ketoglucn$_{in}$ → 2-ketoglucn$_{ext}$	0.003 ± 0.0001	0.003 ± 0.000
F6P$_{in}$ → F6P$_{ext}$	0.001 ± 0.000	0.0001 ± 0.000
G6P$_{in}$ → G6P$_{ext}$	0.001 ± 0.000	0.0001 ± 0.000
R5P$_{in}$ → R5P$_{ext}$	0.002 ± 0.0001	0.000 ± 0.000
α-KG$_{in}$ → α-KG$_{ext}$	0.007 ± 0.005	0.018 ± 0.0003
Fumarate$_{in}$ → Fumarate$_{ext}$	0.000 ± 0.000	0.353 ± 0.495
Malate$_{in}$ → Malate$_{ext}$	0.0004 ± 0.000	0.000 ± 0.000
Succinate$_{in}$ → Succinate$_{ext}$	0.015 ± 0.003	0.008 ± 0.0002

Appendix E

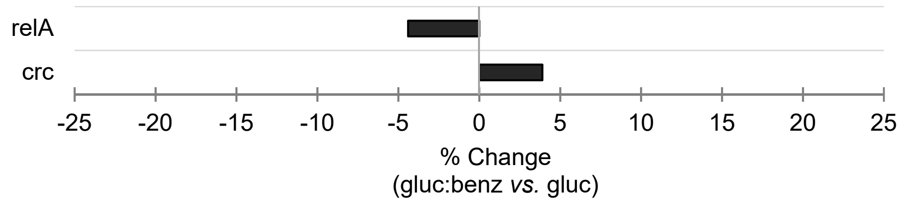
Supplementary Table 4. Fold changes of protein abundance and metabolic fluxes in Pseudomonas putida KT2440 cells grown on glucose:benzoate mixture with respect to cells grown solely on glucose.
Refer to the legends of Fig 1 (main text) for the metabolite abbreviations.

Reactions	ORF number	Protein	Protein abundance	Protein Phosphorylation		Metabolic Flux
			(Glucose:Benzoate)/Glucose			
				Abundance	Site	
Gluc$_{ext}$ → Gluc$_{peri}$	PP_1019	OPRB-1	0.83 ± 0.17	n/a	n/a	0.42 ± 0.002
Gluc$_{ext}$ → G6P	PP_0795	FRUA	1.01 ± 0.14	n/a	n/a	0.43 ± 0.07
Gluc$_{peri}$ → Gluc	PP_1018	GTSD	0.77 ± 0.24	n/a	n/a	0.72 ± 0.50
	PP_1017	GTSC	0.93 ± 0.35	n/a	n/a	0.72 ± 0.50
	PP_1016	GTSB	1.00 ± 0.44	n/a	n/a	0.72 ± 0.50
	PP_1015	GTSA	0.68 ± 0.15	n/a	n/a	0.72 ± 0.50
Gluc$_{peri}$ → G6P	PP_1011	GLK	1.01 ± 0.15	n/a	n/a	0.72 ± 0.50
Gluc$_{peri}$ → Glucn$_{peri}$	PP_1444	GCD	0.92 ± 0.07	n/a	n/a	0.39 ± 0.05
Glucn$_{peri}$ → Glucn	PP_3417	GNTP	0.97 ± 0.25	n/a	n/a	0.43 ± 0.07
Glucn$_{peri}$ → 6-PG	PP_3416	GNUK	0.95 ± 0.25	n/a	n/a	0.43 ± 0.07
Glucn$_{peri}$ → 2KGlucn$_{peri}$	PP_3383	n/a	0.79 ± 0.38	n/a	n/a	0.52 ± 0.08
	PP_3384	n/a	0.77 ± 0.31	n/a	n/a	0.52 ± 0.08
	PP_3382	n/a	0.77 ± 0.37	n/a	n/a	0.52 ± 0.08
2KGlucn$_{peri}$ → 2KGlucn	PP_3377	KGUT	0.79 ± 0.39	n/a	n/a	0.55 ± 0.08
2-KGlucn → 2KGlucnP	PP_3378	KGUK	0.73 ± 0.31	n/a	n/a	0.55 ± 0.08
2KGlucnP → 6-PG	PP_3376	KGUD	0.75 ± 0.23	n/a	n/a	0.55 ± 0.08
G6P → 6-PG	PP_1022	ZWFA	0.92 ± 0.31	n/a	n/a	0.95 ± 0.71
	PP_5351	ZWF	0.88 ± 0.10	n/a	n/a	0.95 ± 0.71
	PP_4042	ZWFB	0.89 ± 0.10	n/a	n/a	0.95 ± 0.71
6-PG → KDPG	PP_1010	EDD	0.97 ± 0.29	n/a	n/a	0.47 ± 0.04
KDPG → Pyruvate + GAP	PP_1024	EDA	0.94 ± 0.24	n/a	n/a	0.47 ± 0.04
GAP → 1,3-bisPG	PP_3443	n/a	0.71 ± 0.11	n/a	n/a	0.10 ± 0.03
	PP_2149	GAP-2	1.04 ± 0.11	n/a	n/a	0.10 ± 0.03
1,3-bisPG → 3PG	PP_4963	PGK	1.09 ± 0.05	n/a	n/a	0.10 ± 0.03
2PG ↔ 3PG	PP_5056	PGM	1.06 ± 0.08	n/a	n/a	-0.10 ± 0.02
2-PG ↔ PEP	PP_1612	ENO	0.96 ± 0.04	n/a	n/a	-0.10 ± 0.02
Pyruvate → PEP	PP_2082	PPSA	0.87 ± 0.07	0.643	T416	-0.05 ± 0.01
PEP → Pyruvate	PP_4301	PYKF	0.92 ± 0.10	n/a	n/a	-0.05 ± 0.01
	PP_1362	PYKA	0.94 ± 0.05	n/a	n/a	-0.05 ± 0.01
DHAP ↔ GAP	PP_4715	TPIA	0.95 ± 0.05	n/a	n/a	1.57 ± 0.34
FBP ↔ DHAP + GAP	PP_4960	FDA	1.06 ± 0.07	n/a	n/a	1.78 ± 0.49
FBP → F6P	PP_5040	FBP	1.09 ± 0.05	n/a	n/a	1.63 ± 0.40
G6P ↔ F6P	PP_4701	PGI2	0.89 ± 0.11	n/a	n/a	1.40 ± 0.91
	PP_1808	PGI1	1.13 ± 0.06	n/a	n/a	1.40 ± 0.91

6-PG → Ru5P + CO_2	PP_4043	GND	0.99 ± 0.12	n/a	n/a	3.19 ± 0.06
Ru5P ↔ R5P	PP_5150	RPIA	1.16 ± 0.06	n/a	n/a	2.86 ± 0.07
Ru5P ↔ Xu5P	PP_0415	RPE	1.03 ± 0.08	n/a	n/a	2.71 ± 0.07
Xu5P + R5P ↔ GAP + S7P	PP_4965	TKTA	1.07 ± 0.05	n/a	n/a	-2.12 ± 0.12
S7P + GAP ↔ E4P + F6P	PP_2168	TAL	0.86 ± 0.12	n/a	n/a	-2.12 ± 0.12
F6P + GAP ↔ E4P + Xu5P	PP_4965	TKTA	1.07 ± 0.05	n/a	n/a	1.82 ± 0.06
Pyruvate → Acetyl-CoA + CO_2	PP_0338	ACEF	0.97 ± 0.10	1.25	S826	0.44 ± 0.11
	PP_0399	ACEE	0.98 ± 0.07	n/a	n/a	0.44 ± 0.11
OAA → Pyruvate + CO_2	PP_1505	PPC	0.85 ± 0.10	n/a	n/a	0.78 ± 0.02
Pyruvate → CO_2 → OAA	PP_5347	OADA	0.87 ± 0.24	n/a	n/a	0.78 ± 0.02
	PP_5346	ACCC2	0.86 ± 0.30	n/a	n/a	0.78 ± 0.02
OAA → PEP + CO_2	PP_1505	PPC	0.85 ± 0.10	n/a	n/a	0.78± 0.09
OAA + Acetyl-CoA → Citrate	PP_4194	GLTA	1.18 ± 0.06	n/a	n/a	0.76 ± 0.11
Citrate → *Cis*-aconitate	PP_2112	ACNA	0.74 ± 0.05	n/a	n/a	0.76 ± 0.11
	PP_2339	ACNB	1.10 ± 0.04	n/a	n/a	0.76 ± 0.11
Cis-aconitate → *Iso*citrate	PP_2112	ACNA	0.74 ± 0.05	n/a	n/a	0.76 ± 0.11
	PP_2339	ACNB	1.10 ± 0.04	n/a	n/a	0.76 ± 0.11
*Iso*citrate → α-KG	PP_4011	ICD	0.65 ± 0.07	n/a	n/a	0.51 ± 0.33
	PP_4012	n/a	1.04 ± 0.05	0.46	S115	0.51 ± 0.33
α-KG → Succinyl-CoA + CO_2	PP_5366	LPD	0.84 ± 0.12	n/a	n/a	0.44 ± 0.37
	PP_4404	IPDV	0.78 ± 0.09	n/a	n/a	0.44 ± 0.37
	PP_4187	LPDG	1.10 ± 0.15	n/a	n/a	0.44 ± 0.37
	PP_4188	SUCB	1.03 ± 0.10	n/a	n/a	0.44 ± 0.37
	PP_4189	SUCA	1.08 ± 0.12	n/a	n/a	0.44 ± 0.37
Succinyl-CoA → Succinate	PP_4185	SUCD	1.05 ± 0.14	n/a	n/a	0.44 ± 0.37
	PP_4186	SUCC	1.06 ± 0.08	n/a	n/a	0.44 ± 0.37
Succinate → Fumarate	PP_4193	SDHC	1.62 ± 0.38	n/a	n/a	2.66 ± 0.18
	PP_4190	SDHB	1.58 ± 0.07	n/a	n/a	2.66 ± 0.18
	PP_4191	SDHA	1.40 ± 0.07	n/a	n/a	2.66 ± 0.18
Fumarate → Malate	PP_1755	FUMC-2	0.84 ± 0.11	n/a	n/a	2.55 ± 0.25
	PP_0944	FUMC	0.82 ± 0.32	n/a	n/a	2.55 ± 0.25
Malate + NAD(P)+ ↔ OAA + NAD(P)H + H+	PP_0654	MDH	1.07 ± 0.13	n/a	n/a	2.54 ± 0.12
Malate + a quinone → oxaloacetate + a quinol	PP_2925	MQO3	0.79 ± 0.14	n/a	n/a	2.54 ± 0.12
	PP_1251	MQO2	0.97 ± 0.06	n/a	n/a	2.54 ± 0.12
	PP_0751	MQO1	1.38 ± 0.30	n/a	n/a	2.54 ± 0.12
Malate → Pyruvate + CO_2	PP_5085	MAEB	0.86 ± 0.07	n/a	n/a	0.69 ± 0.00
Benzoate_{ext} → Benzoate_{peri}	PP_3168	BENF	1.45 ± 0.44	n/a	n/a	n/a
Benzoate_{peri} → Benzoate	PP_3165	BENK	1.15 ± 0.21	n/a	n/a	n/a
Benzoate → Benz-1,2-diol	PP_3163	BENC	1.53 ± 0.42	n/a	n/a	n/a
	PP_3162	BENB	1.60 ± 0.52	n/a	n/a	n/a
	PP_3161	BENA	1.67 ± 0.52	n/a	n/a	n/a
Benz-1,2-diol → Catechol + CO_2	PP_3164	BEND	1.38 ± 0.42	n/a	n/a	n/a

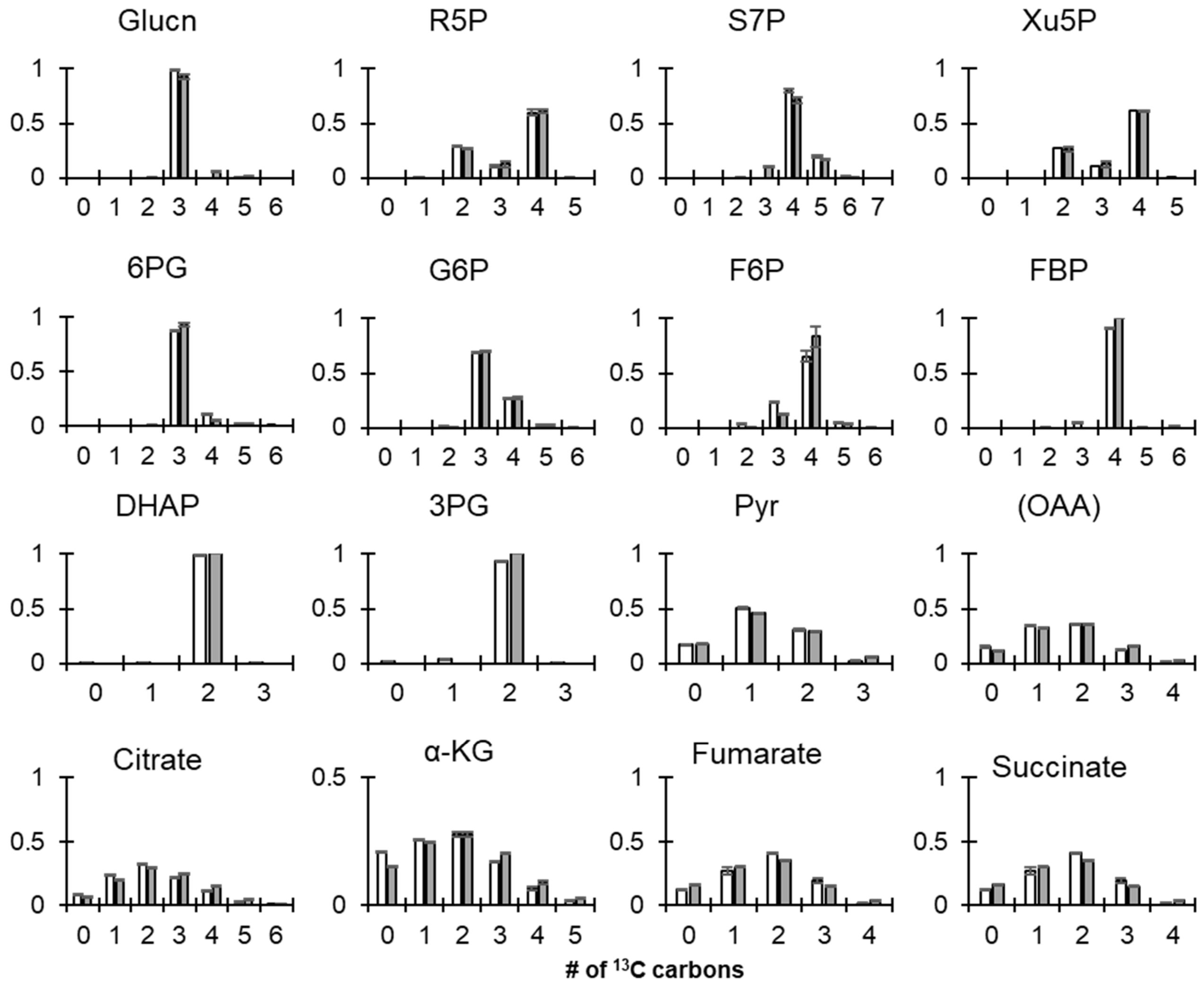
Catechol → *cis,cis*-Muconate	PP_3713	CATA	1.65 ± 0.46	n/a	n/a	n/a
cis,cis-Muconate → Muconolactone	PP_3715	CATB	1.44 ± 0.44	n/a	n/a	n/a
Muconolactone → 4-ketoadipate enol-lactone	PP_3714	CATC	2.03 ± 0.90	n/a	n/a	n/a
4-ketoadipate enol-lactone → 3-ketoadipate	PP_1380	PCAD	1.55 ± 0.46	n/a	n/a	n/a
3-ketoadipate → 3-ketoadipyl-CoA	PP_3952	PCAJ	1.50 ± 0.42	n/a	n/a	n/a
	PP_3951	PCAI	1.29 ± 0.33	n/a	n/a	n/a
3-ketoadipyl-CoA → Succinyl-CoA + Acetyl-CoA	PP_1377	PCAF	1.34 ± 0.33	n/a	n/a	n/a
Citrate → Glyoxylate + Succinate	PP_0356	GLCB	0.83 ± 0.09	n/a	n/a	n/a
Glyoxylate + Acetyl-CoA → Malate	PP_4116	ACEA	0.68 ± 0.14	n/a	n/a	n/a

Appendix F

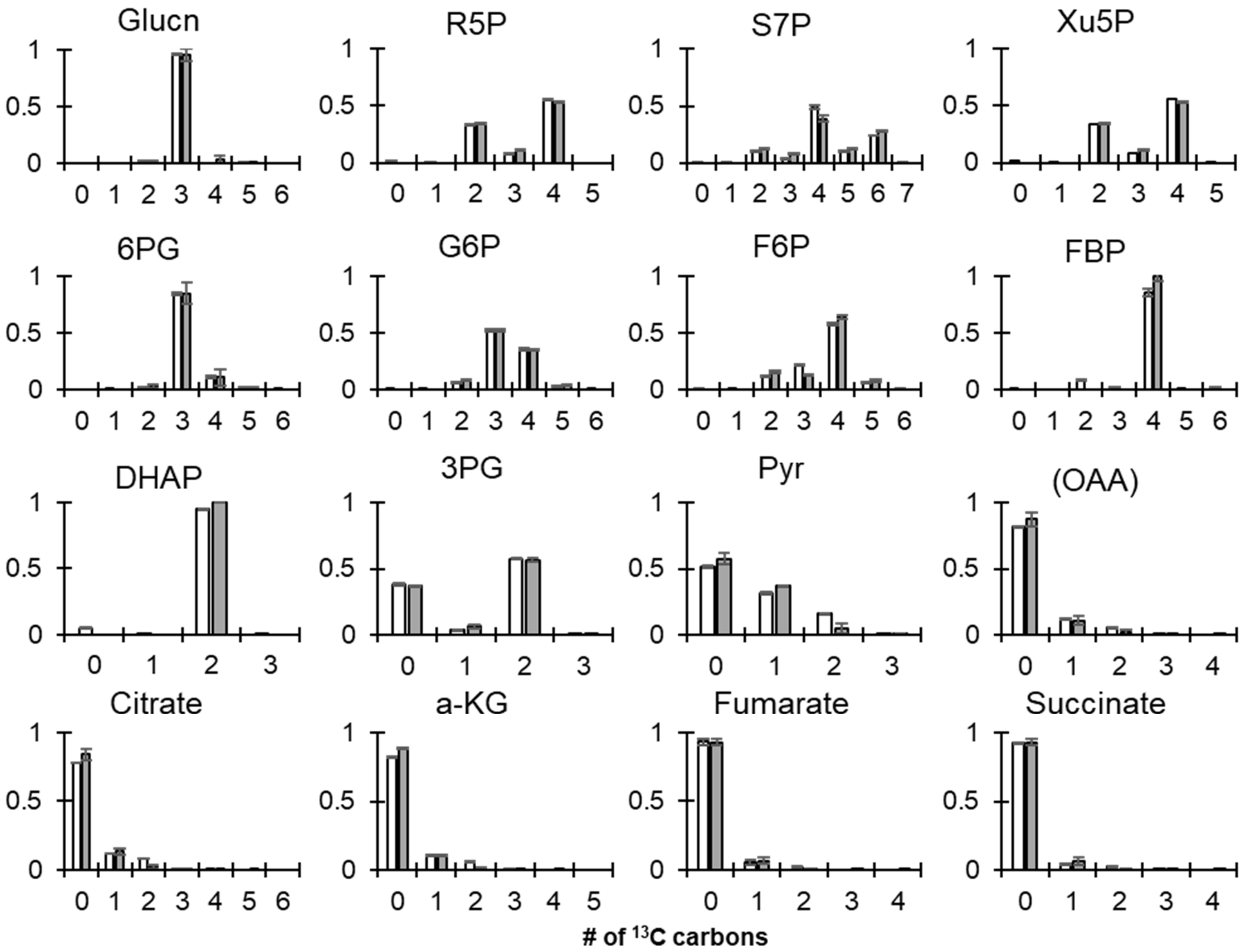


Supplementary Figure 4. Percent change of catabolite repressor proteins by comparing cells fed on both glucose and benzoate to cells fed on glucose alone. Data were obtained from three biological replicates. Note: Only PTSN and CRC, out of five proteins (RELA, CYOB, CRP, PTSN, and CRC) previously investigated for exerting possible catabolite repression in *P. putida* KT2440(del Castillo et al., 2007), were confirmed to play a role in catabolic repression (Ruiz-Manzano et al., 2005; del Castillo et al., 2007): PTSN repressed the pathway for initial aromatic substrate catabolism; CRC repressed reactions in initial glucose catabolism.

Appendix G



Supplementary Figure 5. Experimentally-determined (white bars) and model-estimated (grey bars) of isotopologue distributions in the carbon labeling patterns of selected metabolites in *P. putida* KT2440 cells grown on [1,5,6-$^{13}C_3$]-glucose. Data presented is the average of two independent optimizations of experimental data with two model predictions, both shown with standard deviation error bars. Refer to Fig. 1 (main text) for metabolite abbreviations.



Supplementary Figure 6. Experimentally-determined (white bars) and model-estimated (grey bars) of isotopologue distributions in the carbon labeling patterns of selected metabolites in *P. putida* KT2440 cells grown on [1,5,6-$^{13}C_3$]-glucose with unlabeled benzoate. Data presented is the average of two independent optimizations of experimental data with two model predictions, both shown with standard deviation error bars. Refer to Fig. 1 (main text) for metabolite abbreviations.

References:

Ruiz-Manzano, A., Yuste, L. & Rojo, F. Levels and Activity of the Pseudomonas putida Global Regulatory Protein Crc Vary According to Growth Conditions. *J. Bacteriol.* **187,** 3678–3686 (2005).

Del Castillo, T. & Ramos, J. L. Simultaneous catabolite repression between glucose and toluene metabolism in Pseudomonas putida is channeled through different signaling pathways. *J. Bacteriol.* **189,** 6602–6610 (2007).

Sudarsan, S., Dethlefsen, S., Blank, L. M., Siemann-Herzberg, M. & Schmid, A. The Functional Structure of Central Carbon Metabolism in Pseudomonas putida KT2440. *Appl. Environ. Microbiol.* **80,** 5292–5303 (2014).

Nikel, P. I., Chavarría, M., Fuhrer, T., Sauer, U. & De Lorenzo, V. Pseudomonas putida KT2440 strain metabolizes glucose through a cycle formed by enzymes of the Entner-Doudoroff, embden-meyerhof-parnas, and pentose phosphate pathways. *J. Biol. Chem.* **290,** 25920–25932 (2015).