Molecular Biosystems

Effect of Global Transcriptional Regulators on anaerobic fermentative metabolism of *Escherichia coli*

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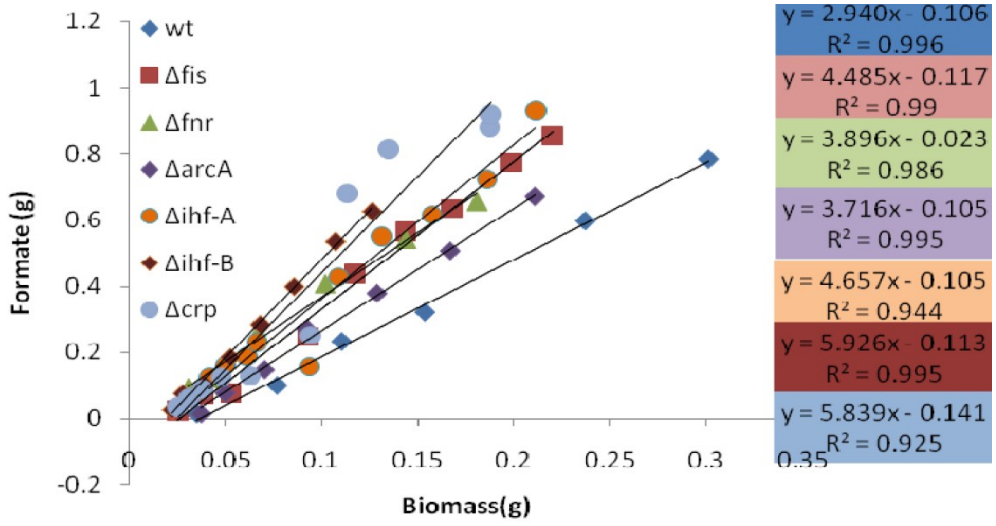

Figure S1: Slopes of concentration of formate versus biomass concentration for WT, *Δfis*, *Δfnr*, *ΔarcA*, *ΔihfA*, *ΔihfB* and *Δcrp*. Note that, the value of this slope gives the yield of formate per biomass, $Y_{g(formate)|g(biomass)}$.

Figure S1 shows the linear relationship between formate concentration and biomass, indicating that formate is a growth associated product. The value of the slope is different for the strains, indicating different carbon distribution for each strain.

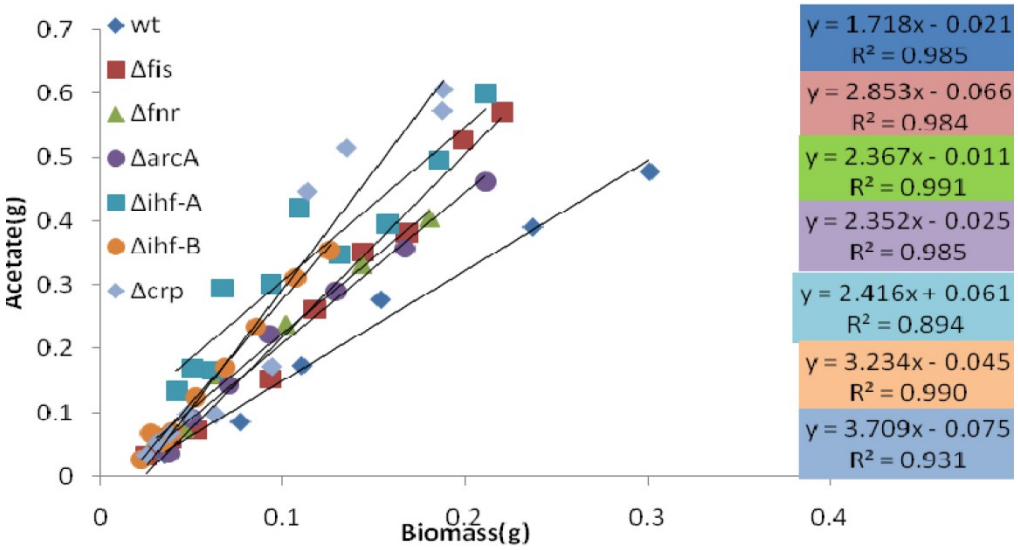

Figure S2: Slopes of concentration of acetate versus biomass concentration WT, *Δfis*, *Δfnr* , *ΔarcA, ΔihfA, ΔihfB* and *Δcrp*. Note that, the value of this slope is the yield of acetate per biomass, $Y_{g(acetate|g(biomass)}$.

Figure S2 shows that acetate concentration has a linear relationship with biomass, indicating that acetate is a growth associated product. The slope for all the strains is different from each other, indicating different carbon distribution towards acetate for each strain.

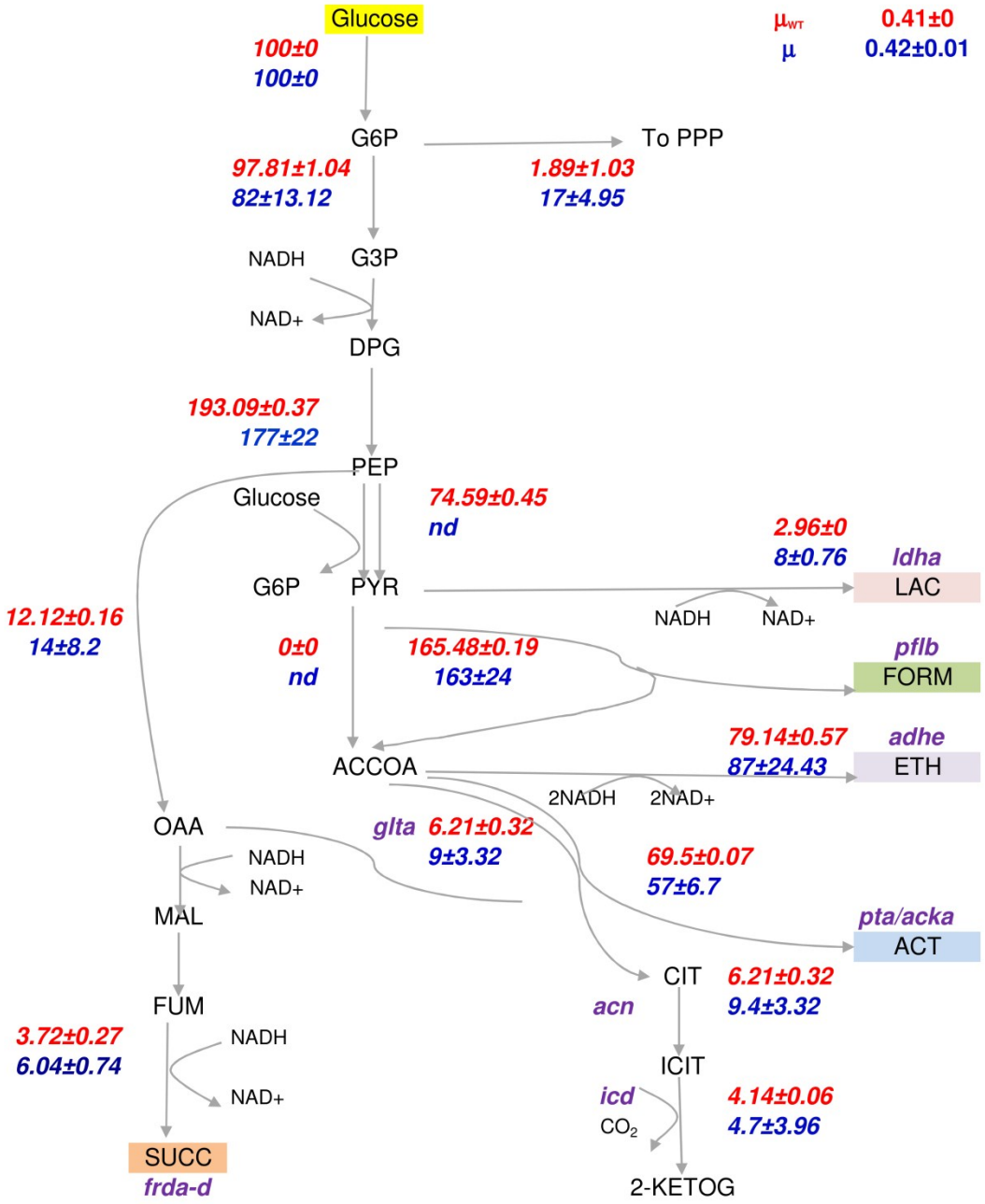

Figure S3: Flux distribution in the central carbon metabolism in the WT strain under anaerobic fermentation conditions. Note that the fluxes are normalized with glucose uptake rate (16.95 mM/g(dcw)*h). The flux values on the top are from current study and the bottom are as reported by 13-C study in Chen *et al* .[22] Chen *et al.,* have reported the glucose uptake rate to be (14.9 mM/g(dcw)*h)) . The mixed acids are abbreviated as LAC for lactate, FORM for formate, ACT for acetate, ETH for ethanol and SUCC for succinate

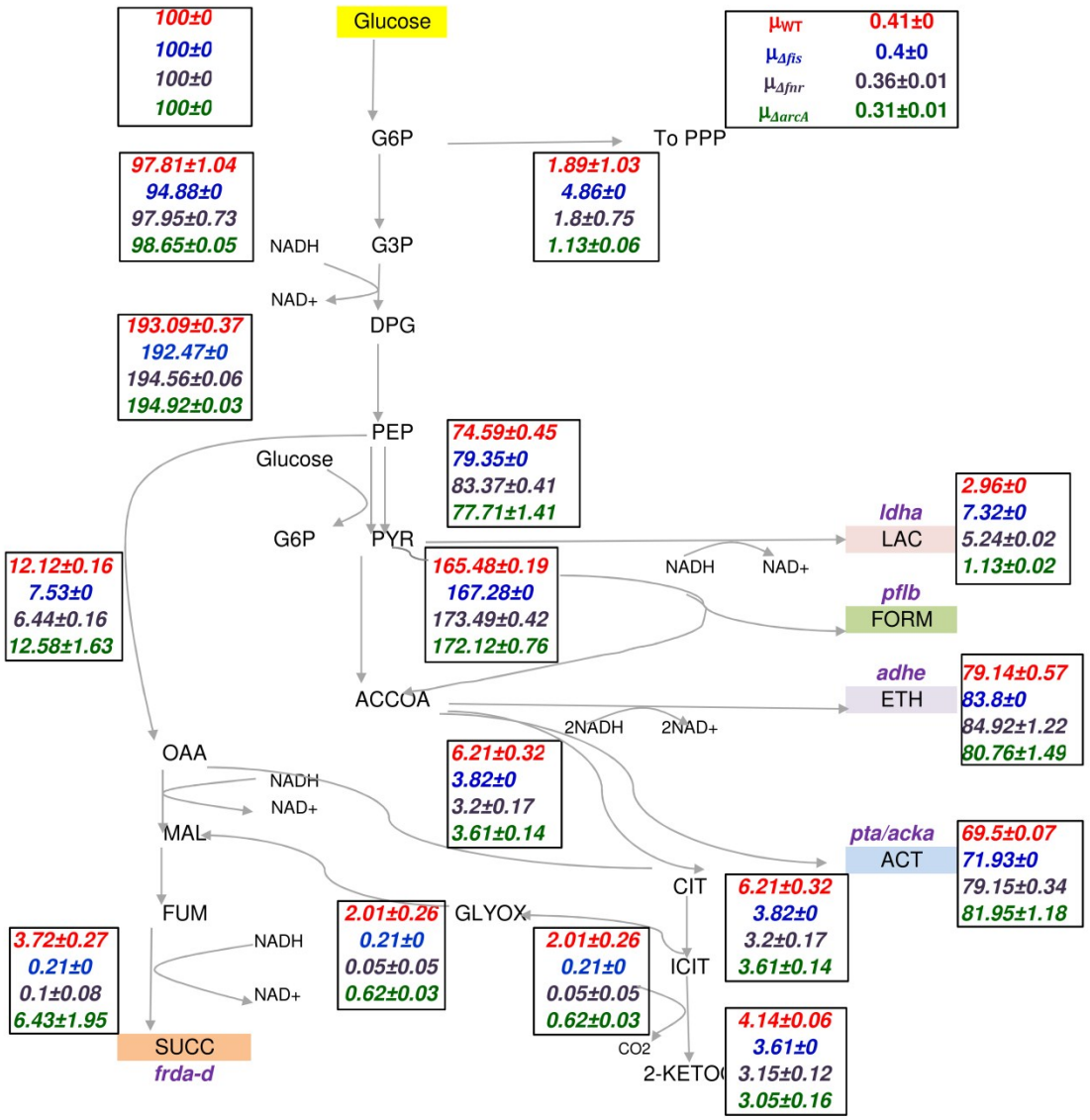

Figure S4 : Flux distribution in the central carbon metabolism in the WT, *Δfis, Δfnr,* and *ΔarcA* strain under anaerobic fermentation conditions, from top to bottom. Note that the fluxes are normalized with respective glucose uptake rate for each strain. The mixed acids are abbreviated as LAC for lactate, FORM for formate, ACT for acetate, ETH for ethanol and SUCC for succinate.

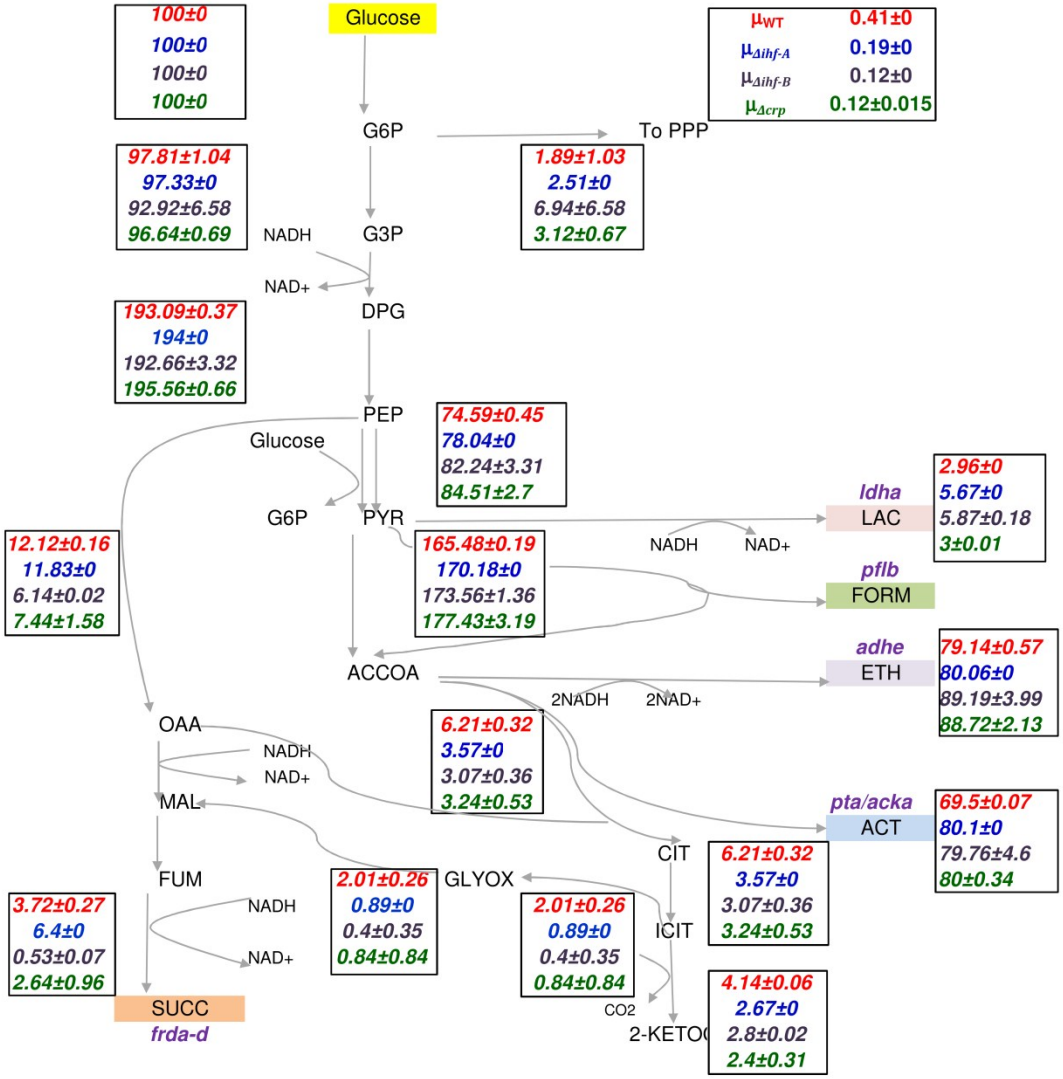

Figure S5 : Flux distribution in the central carbon metabolism in the WT, *Δihf-A, Δihf-B Δcrp* strain under anaerobic fermentation conditions, from top to bottom. Note that the fluxes are normalized with respective glucose uptake rate for each strain. The mixed acids are abbreviated as LAC for lactate, FORM for formate, ACT for acetate, ETH for ethanol and SUCC for succinate.

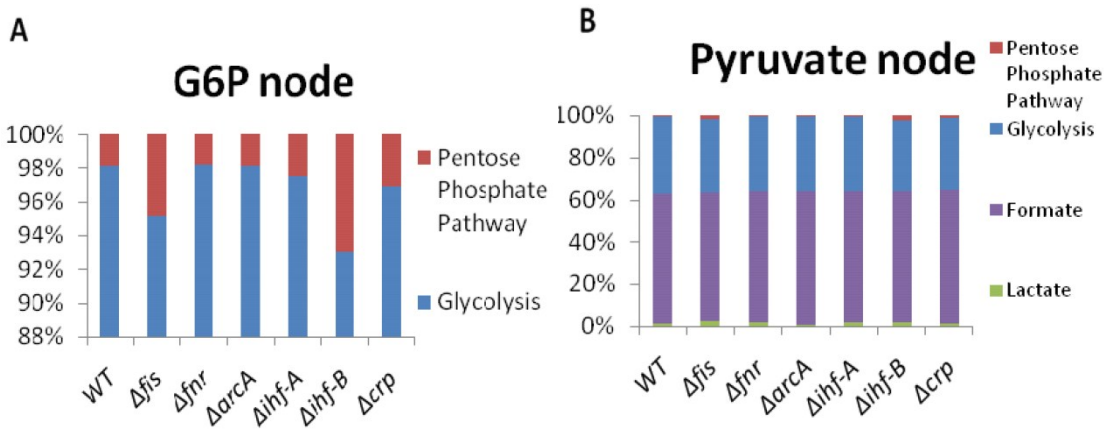

Figure S6: (A) Distribution of fluxes at G6P node in WT and six global transcription factor deletion mutants. A variation of maximum 4 % is noted, indicating the robustness of this node. (B) Distribution of fluxes at pyruvate node. The major part of the fluxes through this node is channeled towards glycolysis and formate production and the various was subtly different.

The distribution of carbon fluxes on these nodes is similar for all the strains, indicating the robustness of these strains and also indicating that the deletion of global transcription factors may not affect the distribution of fluxes. Rather, deletion can affect the absolute fluxes of the metabolites.

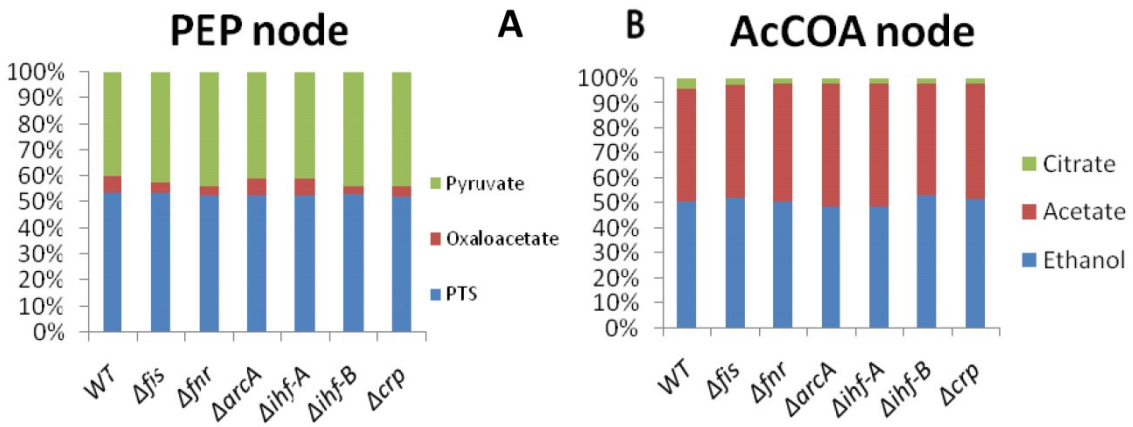

Figure S7: (A) Distribution of fluxes at PEP node in WT and six global transcription factor deletion mutants. The major carbon flux at this node is directed towards pyruvate and PTS branches (B) Distribution of fluxes at AccoA node. The major part of the fluxes through this node is channeled towards acetate and ethanol production.

The distribution of carbon fluxes on these nodes is similar for all the strains, indicating the robustness of these strains and also indicating that the deletion of global transcription factors may not affect the distribution of fluxes. Rather, deletion can affect the absolute fluxes of the metabolites.

Regulation of Central carbon Metabolism genes by transcription factors under study

Global regulators	Genes	
	Positive influence	**Negative influence**
CRP	*ptsG* [2], *ptsH* [8], *gltA* [1] , *acnA* [1], *acnB, icdA* [1], *sdhCDAB* [1], *cyo* [9], *fumAC, acs* [9], *gap* [9], *aceEF* [9], *pdhR* [9], *lpdA , fumB, cyo, pdhR, ackA* [10], *fba* [11], *gapA* [11], *pgk* [11]	*cya, fis, aceBA* [9], *crp-cAMP* [15]
Fis	*ptsG, adhE, hyaA-F* [9], *nuoA-N, ndh, lpd*[15]	*ptsG, fis*, *crp* [6], (cAMP) *cya* [6], *acnB* [9], *acs* [9], *fumB, aldB, pflB*[15]
FNR	*foc-pflB* [2], *frd* [2], *arcA* [2], *adhE* [12], *yfiD* [3], *fumB* [3], *aspA* [3], *pykA* [3]	*cyd* [2], *cyoAE* [2,12], *gltA* [1] , *acnA* [1], *icdA* [1], *fumAC* [1,12], *ndh* [5], *nuoE* [5], *aceBA* [9], *sucABCD* [12], *sdhCDAB* [12], *yfiD* [12]
ArcA	*focA-pflB* [2], *cyd* [2], *frd* [4], *hyaA-F* [9], *fumB, hyaA-1* [12], *yfiD* [12], *appBC* [12],	*fnr*, *ptsG*, *cyo*[2], *pdh* [2], *gltA*[2] , *acnAB,* [2,12], *icdA*[2], *sucABCD* [2], *sdhCDAB* [2,14], *fumA* [2], *mdh* [2], *aceBAKEF* [1], *aceEF-lpd*[1,14], *pykA* [3], *ldh, acs* [9], *glcDEFG* [9], *ndh* [12]
IHF	*fis, fnr, aceBAK* [9], *glcDEFG* [9], *hycA-I* [9] *gltA, focA-pflB*[15]	*pta* [7], *ack* [7], *sucABCD* [9], *ndh, nuoA, sucABCD*[15]

Table S1: This table gives an account of central carbon metabolism genes induced and repressed by global transcription factors studied.

Stoichiometry	Reactions
0.14176 Glyc3P + 26.2949 ATP + 0.60097 Ala + 0.10124 Cys + 0.26647 Asp + 0.30747 Glu + 0.2048 Phe + 0.67725 Gly + 0.10473 His + 0.32116 Ile + 0.37935 Lys + 0.49804 Leu + 0.16989 Met + 0.26647 Asn + 0.24436 Pro + 0.29091 Gln + 0.32698 Arg + 0.38031 Ser + 0.28044 Thr + 0.46778 Val + 0.062835 Trp + 0.15244 Tyr + 0.1489 rATP + 0.18319 rGTP + 0.11366 rCTP + 0.12273 rUTP + 0.023904 dATP + 0.024582 dGTP + 0.024582 dCTP + 0.023904 dTTP + 0.28352 avg_FS + 0.0069264 UDPGlc + 0.010368 CDPEth + 0.010368 OH_myr_ac + 0.010368 C14_0_FS + 0.010368 CMP_KDO + 0.010368 NDPHep + 0.0069264 TDPGlcs + 0.01656 UDP_NAG + 0.01656 UDP_NAM + 0.01656 di_am_pim + 0.0924 ADPGlc ==>	mue
==> O2	O2_up
==> N	N_up
CO2 <==>	CO2_ex
4 ATP + 4 NADPH ==> S	S_up
PEP ==> G6P + Pyr	Glc_PTS_up
ATP ==> G6P	Glc_ATP_up
Succ ==>	Succ_ex
==> Glyc	Glyc_up
ATP + Glyc ==> Glyc3P	Glyc::Glyc3P
DHAP + NADH <==> Glyc3P	DHAP::Glyc3P
Lac ==>	Lac_ex
Eth ==>	Eth_ex
Ac ==>	Ac_ex
==> Glucn	Glucn_up
Form ==>	Form_ex
G6P <==> F6P	G6P::F6P
F16P ==> F6P	F16P::F6P
F6P + ATP ==> F16P	F6P::F16P
F16P <==> DHAP + G3P	F16P::T3P
DHAP <==> G3P	DHAP::G3P
G3P <==> DPG + NADH	G3P::DPG
DPG <==> 3PG + ATP	DPG::3PG

3PG <==> 2PG	3PG::2PG
2PG <==> PEP	2PG::PEP
PEP ==> Pyr + ATP	PEP::PYR
Pyr + 2 ATP ==> PEP	Pyr::PEP
Pyr ==> AcCoA + NADH + CO2	PYR::AcCoA
AcCoA + OxA ==> Cit	AcCoA::Cit
Cit <==> ICit	Cit::ICit
ICit <==> alKG + NADPH + CO2	ICit::alKG
alKG ==> SuccCoA + NADH + CO2	alKG::SuccCoA
SuccCoA <==> Succ + ATP	SuccCoA::Succ
Succ ==> Fum + QuiH2	Succ::Fum
Fum + QuiH2 ==> Succ	Fum::Succ
Fum <==> Mal	Fum::Mal
Mal <==> OxA + NADH	Mal::OxA
ICit ==> Succ + Glyox	ICit::Glyox
AcCoA + Glyox ==> Mal	Glyox::Mal
G6P <==> PGlac + NADPH	G6P::PGlac
AcCoA + NADH <==> Adh	AcCoA::Adh
NADH + Adh <==> Eth	Adh::Eth
PGlac ==> PGluc	PGlac::PGluc
Glucn + ATP ==> PGluc	Glucn::PGluc
PGluc ==> Rl5P + NADPH + CO2	PGluc::Rl5P
Rl5P <==> X5P	Rl5P::X5P
Rl5P <==> R5P	Rl5P::R5P
R5P + X5P <==> G3P + S7P	Transket1
G3P + S7P <==> F6P + E4P	Transaldo
E4P + X5P <==> F6P + G3P	Transket2
PGluc ==> KetoPGluc	PGluc::KetoPGluc
KetoPGluc <==> G3P + Pyr	KetoPGluc::G3P_Pyr
OxA + ATP ==> PEP + CO2	OxA::PEP
PEP + CO2 ==> OxA	PEP::OxA
AcCoA <==> AcP	AcCoA::AcP

AcP <==> ATP + Ac	AcP::Ac
Pyr ==> AcCoA + Form	Pyr::Form
Pyr + NADH <==> Lac	Pyr::Lac
NADH <==> QuiH2 + 2 H_ex	NADHDehydro
QuiH2 + 0.5 O2 ==> 2 H_ex	Oxidase
NADH + H_ex <==> NADPH	TransHydro
3 H_ex <==> ATP	ATPSynth
ATP ==>	ATPdrain
2 PEP + E4P + ATP + NADPH ==> Chor	Chor_Synth
R5P + 2 ATP ==> PRPP	PRPP_Synth
ATP + NADPH <==> MTHF	MTHF_Synth
Pyr + Glu ==> alKG + Ala	Ala_Synth
2 Pyr + NADPH + Glu ==> alKG + CO2 + Val	Val_Synth
2 Pyr + AcCoA + NADPH + Glu ==> alKG + NADH + 2 CO2 + Leu	Leu_Synth
2 ATP + N + Asp ==> Asn	Asn_Synth
OxA + Glu ==> alKG + Asp	Asp_synth
di_am_pim ==> CO2 + Lys	Lys_Synth
SuccCoA + ATP + 2 NADPH + MTHF + Cys + Asp ==> Pyr + Succ + N + Met	Met_Synth
2 ATP + 2 NADPH + Asp ==> Thr	Thr_Synth
Pyr + NADPH + Glu + Thr ==> alKG + CO2 + N + Ile	Ile_Synth
ATP + PRPP + Gln ==> alKG + 2 NADH + His	His_Synth
alKG + NADPH + N ==> Glu	Glu_synth
ATP + N + Glu ==> Gln	Gln_Synth
ATP + 2 NADPH + Glu ==> Pro	Pro_Synth
AcCoA + 4 ATP + NADPH + CO2 + N + Asp + 2 Glu ==> alKG + Fum + Ac + Arg	Arg_Synth
Chor + PRPP + Gln + Ser ==> G3P + Pyr + CO2 + Glu + Trp	Trp_Synth
Chor + Glu ==> alKG + NADH + CO2 + Tyr	Tyr_Synth
Chor + Glu ==> alKG + CO2 + Phe	Phe_Synth
3PG + Glu ==> alKG + NADH + Ser	Ser_Synth
Ser ==> MTHF + Gly	Gly_Synth
AcCoA + S + Ser ==> Ac + Cys	Cys_Synth
5 ATP + CO2 + PRPP + 2 MTHF + 2 Asp + Gly + 2 Gln ==> 2 Fum + NADPH + 2 Glu + rATP	rATP_Synth

6 ATP + CO2 + PRPP + 2 MTHF + Asp + Gly + 3 Gln ==> 2 Fum + NADH + NADPH + 3 Glu + rGTP	rGTP_Synth
ATP + Gln + rUTP ==> Glu + rCTP	rCTP_Synth
4 ATP + N + PRPP + Asp ==> NADH + rUTP	rUTP_Synth
NADPH + rATP ==> dATP	dATP_Synth
NADPH + rGTP ==> dGTP	dGTP_Synth
NADPH + rCTP ==> dCTP	dCTP_Synth
2 NADPH + MTHF + rUTP ==> dTTP	dTTP_Synth
8.24 AcCoA + 7.24 ATP + 13.91 NADPH ==> avg_FS	avg_FS_Synth
G6P + ATP ==> UDPGlc	UDPGlc_Synth
3PG + 3 ATP + NADPH + N ==> NADH + CDPEth	CDPEth_Synth
7 AcCoA + 6 ATP + 11 NADPH ==> OH_myr_ac	OH_myr_ac_Synth
7 AcCoA + 6 ATP + 12 NADPH ==> C14_0_FS	C14_0_FS_Synth
PEP + R5P + 2 ATP ==> CMP_KDO	CMP_KDO_Synth
1.5 G6P + ATP ==> 4 NADPH + NDPHep	NDPHep_Synth
F6P + 2 ATP + N ==> TDPGlcs	TDPGlcs_Synth
F6P + AcCoA + ATP + Gln ==> Glu + UDP_NAG	UDP_NAG_Synth
PEP + NADPH + UDP_NAG ==> UDP_NAM	UDP_NAM_Synth
Pyr + SuccCoA + ATP + 2 NADPH + Asp + Glu ==> alKG + Succ + di_am_pim	di_am_pim_Synth
G6P + ATP ==> ADPGlc	ADPGlc_Synth

Table S2: This table gives an account of reactions used in flux balance analysis (adopted from Klamt *et al.*)[30]

Accumulation rates of metabolites in mid exponential phase

Rates(mM/(g(dcw)*h)	Acetate	Formate	Lactate	Succinate	Ethanol	Glucose
WT	11.91±0.09	26.25±0.25	0.5±0	1.18±0.06	13±0	16.95±0.05
Δ*fis*	15±0	32±0	0.91±0.5	0.3±0	15.7±0	20±0
Δ*fnr*	15.99±0.01	34.25±0.25	1.05±0	0.23±0	16.67±0.67	20.43±0.78
Δ*arcA*	14.4±0.3	30.5±0.5	0.5±0	1.42±0.37	14.3±0.09	17.4±0.4
Δ*ihf-A*	10±0	21±0	0.7±0	1.01±0	7.22±0.02	12.25±0.25
Δ*ihf-B*	5.7±0.3	12.5±0.5	0.44±0.01	0.13±0.02	7.4±0.6	7.5±0
Δ*crp*	7.6±0.1	14.78±0.22	0.18±0.04	0.36±0.01	9.88±0.12	10.25±0.25

Table S3: Accumulation rates of various metabolite production and glucose consumption in mid exponential phase for WT and six global transcription deletion mutants. The above rates were used for flux balance analysis for each strain. The analysis indicated that carbon channeling through different metabolic fluxes is subtly regulated by transcription factors through metabolic enzymes. Note that, all the rates are represented as mM/(g(dcw)*h).

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