SUPPLEMENTARY NOTES

Discussion of Prism modules and predicted interactions (Fig. 4)

a. Interactions of the TCA-cycle, respiratory chain, and ATP synthetase with the amino acid biosynthesis modules. Given the input conditions of low oxygen and *RIP1* deletion used for FBA, overall respiratory function is low in the wild type network. This is consistent with the fact that *S. cerevisiae* has low catabolic fluxes through the TCA cycle which at maximum growth rates operates as a bifurcated pathway to fulfil exclusively biosynthetic functions[19,20]. Accordingly, the doubly perturbed *ACO1-YJL200C* network (aconitate hydratase isoenzymes, EC 4.2.1.3) cannot yield biomass because the main pathway generating α-ketoglutarate for amino acid biosynthesis is blocked. Lysine and proline biosynthesis are good examples of the interactions resulting from the connection between carbon catabolism and amino acid biosynthesis. In yeast, lysine is synthesized through the α-aminoadipate pathway that uses α-ketoglutarate and acetyl-CoA [21]. Similarly, proline biosyntheis also requires α-ketoglutarate, which in this case is channeled through glutamate synthesis. *KGD2* and *LSC2* appear co-lethal with amino acid metabolism because of the common substrates of these TCA-cycle enzymes with lysine biosynthesis. In other words, mutants incapable of metabolizing α-ketoglutarate through the TCA-cycle cannot longer grow when flux of this metabolite through lysine or proline biosynthesis is additionally blocked.

Both lysine and proline biosynthesis pathways require reduced NADPH and are at least partially compartmentalized in the mithochondria. Consequently, mutations in the NADPH-yielding *IDP1* enzyme (NADP-isocitrate dehydrogenase, EC 1.1.1.42) are completely buffered with those of any of the lysine biosynthesis genes[22] (see **Supplementary Fig. 4a**). Similarly, genes of the proline synthesis pathway which use also requires reduced NADPH, show buffering links within each other and with the disrupted *IDP1*-encoded enzyme.

b. Interactions within and beyond the glycolysis module. Several aggravating interactions of the glycolysis module are observed in **Fig. 4a**. Biomass production cannot

be sustained when enzymes of the pentose phosphate pathway or gluconeogenesis, which are part of a given bypass, are further perturbed. On the other hand, ATP synthesis relies now on oxidative phosphorylation, and thus disrupted glycolysis is aggravated by alteration of respiratory chain genes or the ATP synthetase. Since substrate-level phosphorylation is not anymore the source of ATP in the perturbed network, the process of ethanol transport shows a buffering link with *PGK1* (3-phosphoglycerate kinase, EC 2.7.2.3) and *CDC19*-encoded enzymes, reflecting that altering yeast fermentative properties cannot further increment the deleterious effect of perturbing any of the two ATP-generating glycolytic enzymes. Together with the numerous aggravating links, some buffering interactions are also observed within the glycolysis module, reflecting its unbranched character. In agreement with this, *PFK1* (phosphofructokinase, EC 2.7.1.11) and *CDC19* account for all the buffering interactions appearing in the glycolysis module, because these are the two genes involved in the non-reversible steps of glycolysis and thus the directionality of the pathway.

c. Interactions of the respiratory chain module with the ATPs module. Perturbation of complexes I, II, or III has no detectable effect in the simulation because of the additional constraint set by eliminating the flux through complex III (*RIP1*). Nevertheless, flux through complex IV is still relevant due to the presence of *NCP1* and U109, which together form an electron flux pathway from reduced NADPH to O_2. Within this context, combining double deletions of *COX1* (cytochrome c oxidase), *NCP1* (NADPH-ferrihemoprotein reductase), and U109 (O_2 passive transport to the mitochondria) results in a collection of non-directional buffering links within the respiratory chain module, as this elements are part of the same linear electron flux pathway from reduced NADPH to O_2. However, the whole module displays a directional buffering link with the ATP synthetase, because electron transport through complex IV is coupled to the pumping of protons that are substrate for ATP synthesis through *ATP8* (see **Supplementary Fig. 4b**). In other words, disruption of the ATP synthetase module affects exclusively ATP synthesis through oxidative phosphorilation, while perturbation of the respiratory chain module affects both ATP synthesis and cofactor oxidation, thus having an added effect on the flux of NADP requiring pathways.

d. Aggravating links between lysine biosynthesis and tryptophan catabolism. Unexpectedly, the lysine biosynthesis pathway clearly shows aggravating interactions with the tryptophan degradation pathway, which constitutes the *de novo* pathway for NAD^+ biosynthesis via kynurenine[23]. Although no experimental connection between this route with that of lysine biosynthesis has been experimentally detected in *S. cerevisiae*, the reconstructed metabolic network does include aminocarboxymuconate-semialdehyde decarboxylase (EC 4.1.1.45) and aminomuconate-semialdehyde dehydrogenase (EC 1.2.1.32), which together convert a-amino-3-carboxymuconate semialdehyde to α-ketoadipate, intermediates of the NAD^+ and lysine biosynthesis pathways, respectively. Thus, the first steps of lysine biosynthesis can be bypassed through tryptophan degradation resulting in the observed interaction between these routes. Consistent with this, only those enzymes that yield α-ketoadipate in the lysine pathway (*LYS20*, *LYS4*, and *LYS12*) display aggravating links with the metabolism of tryptophan, whereas every enzyme participating in the conversion of α-ketoadipateto lysine (*LYS2*, *LYS9*, and *LYS1*) is essential for growth in minimal medium (see **Supplementary Fig 4a**).

e. Interactions of the Proline metabolism modules. Proline biosynthesis shows an aggravating interaction with the respiratory chain, due to the presence of alternative routes for proline biosynthesis. The primary proline biosynthesis pathway can be bypassed through the interconversion of arginine and ornithine by the action of the *CAR2*-encoded ornithine aminotransferase (EC 2.6.1.13) which yields glutamate semialdehyde[24]. In agreement with this, the first steps of the route encoded by *PRO1* (glutamate-5 kinase, EC 2.7.2.11) and *PRO2* (glutamate-5-semialdehyde dehydrogenase, EC 1.2.1.41) appear co-lethal with *CAR2*, while disruption of the *PRO3*-encoded enzyme (Δ-1-pyrroline-5-carboxylate reductase, EC 1.5.1.2) that converts glutamate semialdehyde to proline is unviable by itself. However, the need of an intact TCA cycle and respiratory chain for the interconversion of asparagine, proline, and ornithine, has not been observed. Also, the buffering interaction between the primary proline biosynthesis route and the ATP synthetase were not expected and may reflect the ATP requirement of this mitochondrial pathway.

It should be stressed that under the fermentative growth conditions considered for FBA, the role of respiratory chain in oxidative phosphorylation is not predominant, but still *COX1* disruption aggravates perturbation of proline catabolism module. Proline degradation occurs through the action of the FAD^+-dependent *PUT1*-encoded proline dehydrogenase (EC 1.5.99.8) and the NAD^+-utilizing Δ-1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12)[25]. Therefore, a crosstalk between the cycling of oxidized and reduced forms of FAD^+ and NAD^+ in the reconstructed metabolic network could account for the aggravating link between these processes.

f. Effect of single mutations in glycolytic enzymes. Perturbation of non-redundant glycolytic enzymes results in a strong reduction of biomass production under the FBA framework, but not in a complete lack of growth. This is inconsistent with the experimental observation that most knockout mutants lacking glycolysis enzymes are not viable when grown on glucose as sole carbon source[26]. For example, although yeast lacks a major bypass of the lower reactions of glycolysis between triose phosphates and pyruvate[27], the *CDC19*-perturbed network (pyruvate kinase, EC 2.7.1.40) still produces biomass under FBA. A close inspection to the fluxes in the perturbed network shows that carbon flux is bypassed through the biosynthesis and degradation of serine and tryptophan. Optimization in FBA makes use of the fact that anabolism and catabolism of these compounds follow different routes which on one hand use glycolysis intermediates and on the other produce pyruvate and TCA cycle intermediates. We are thus aware that certain interactions unavoidably result from the FBA model as opposed to the fluxes that would be expected *in vivo*, but that nevertheless are consistent within the model. In this regard, such large flux redistributions would not be allowed by the Minimization Of Metabolic Adjustment (MOMA) algorithm[13].

g. Genetic redundancy in the reconstructed metabolic network. One of the limitations of predicting phenotypes and genetic interactions through FBA is related to the prevalence of biochemical reactions encoded by multiple genes in *S. cerevisiae*, namely isoenzymes, whose potential differential regulation cannot be taken into account.

In certain instances, the predicted synthetic lethality of duplicated genes is consistent with knowledge resulting from experimental research. This is the case of *TKL1* and *TKL2*; either of the transketolase isoenzymes (EC 2.2.1.1) they encode must be intact for the generation of erythrose 4-phosphate and therefore necessary for the biosynthesis of aromatic amino acids[28]. However, several false-negative interactions are to be expected where duplicated genes merely appear as synthetic lethals, but could play particular roles due to the ubiquitous differential regulation of isoenzymes[29]. As an example, the *PGM1*-perturbed network shows no change with respect to wild type, although mutants are known to be lethal even though yeast possesses two additional phosphoglucomutase isoenzymes[30]. In a different case, the deleterious effect of mutations of the *GDH1*-encoded glutamate dehydrogenase should uncover the known interaction of this enzyme with the TCA-cycle enzymes[31], but are hidden in FBA because of the existence of the *GDH3* homologue whose repression by glucose is ignored in the reconstructed network. Still, differential localization of mitochondrial and non-mitochondrial isoforms is considered for FBA, uncovering interesting interactions as those previously described for *IDP1*. However, the analysis of this particular case is further limited by the existence of cytosolic and peroxisomal $NADP^+$-dependent isocitrate dehydrogenase isoenzymes encoded by *IDP2* and *IDP3*, respectively[32], which are predicted to be co-lethal.

(For references, see **Supplementary References** online)