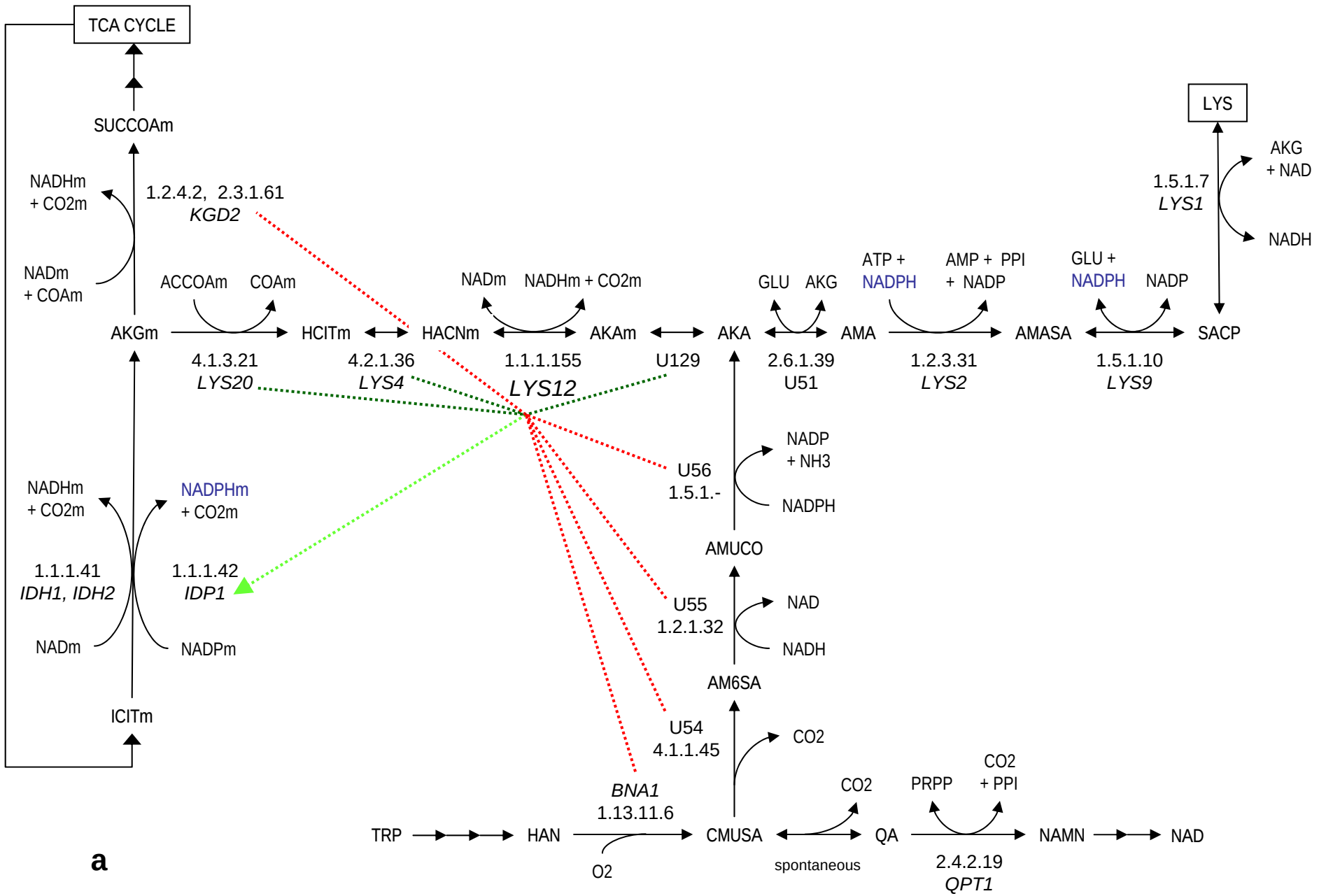
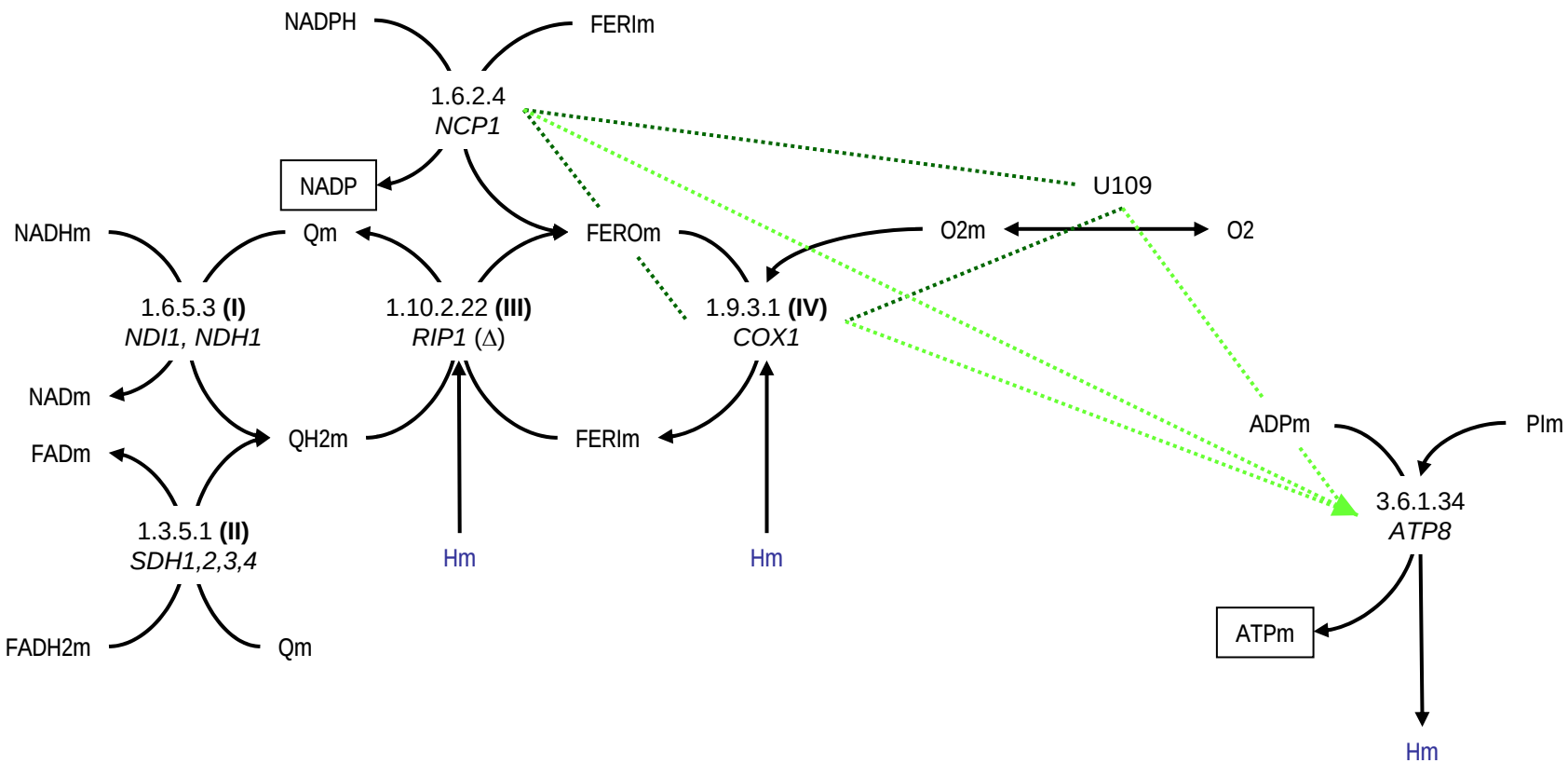
Supplementary Figure 4



a


NADPH
FERIm
1.6.2.4
NCP1
NADP
U109
NADHm
Qm
FEROm
O2m
O2
1.6.5.3 (I)
NDI1, NDH1
1.10.2.22 (III)
RIP1 (Δ)
1.9.3.1 (IV)
COX1
NADm
FADm
QH2m
FERIm
ADPm
PIm
3.6.1.34
ATP8
1.3.5.1 (II)
SDH1,2,3,4
Hm
Hm
ATPm
FADH2m
Qm
Hm

b

Supplementary Fig. 4: Sub-classification of buffering interactions into directional and non-directional links is related to the underlying biochemical network. A non-directional (symmetrical) link exists when each of the single mutations and the double deletion exhibit the same effect; while directional (asymmetrical) links are formed when the effects of the single mutations are not equal and the mutation with the stronger effect buffers the other one. These asymmetric links could thus be described as a directed arrow pointing from the buffering to the buffered mutation. While non-directional interactions most often reflect trivial buffering between genes belonging to the same serial pathway, directional links suggest more complex interactions, normally between genes which have different, yet related, functions in the network. Of the total number of buffering interactions scored with FBA in the yeast metabolic network, a significant proportion (63%) consists of such non-trivial asymmetric links. In this figure, as throughout the work, this subtle distinction between directional and non-directional buffering interactions was not used as an input for the Prism algorithm. It is therefore intriguing to see that, while being blind to such sub-classification, the Prism algorithm results in monochromatically interacting modules that are characterized by non-directional intra-module links and directional inter-module links (**Fig. 4a**). Here we show in detail a portion of the metabolic network and the ensuing epistatic links. **(a)** Interactions of the lysine biosynthesis module, demonstrating the qualitative difference between directional and non-directional buffering links. Aggravating (dotted red lines), non-directional buffering (dark green dotted lines), and directional buffering (dotted light green directional arrows) interactions of *LYS12* are shown. In yeast, lysine (LYS) is synthesized through the α-aminoadipate (AMA) pathway, which uses α-ketoglutarate (AKG) and acetyl coenzyme-A (ACCOA) as carbon precursors. The model predicts that mutants in any of the first four steps of the pathway are not complete auxotrophs because of the connection of this route with tryptophan (TRP) degradation via α-ketoadipate (AKA). In other words, TRP catabolism functions as an ancillary pathway when any of the AKA-yielding genes are disrupted. As expected, the combination of *LYS12* deletion with *LYS20*, *LYS4*, or U129 results in buffering non-directional links because these elements are part of the same serial pathway. As opposed to this, the NADP-dependent isocitrate dehydrogenase (*IDP1*) displays a directional buffering interaction with *LYS12*. The fact

that reduced NADPH is required for lysine production results in a link in which *IDP1* deletion is completely buffered in a LYSbs-null background. Finally, *KGD1* or *LSC2* deletion will aggravate the effect of perturbing *LYS12*, presumably because AKG cannot be further metabolized through LYS biosynthesis when the TCA cycle is impaired. **(b)** Interactions of the respiratory chain module with the ATPs module, displaying a more complex subdivision of buffering links into directional and non-directional. 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*. 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. 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. The gene and metabolite names are from the FBA reaction list[15], accessible at http://www.cpb.dtu.dk/models/yeastmodel.html .

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