

Saccharomyces cerevisiae LEU9 MYKHSFIALAEHA-SKLRSIPPVYKLTYNHLRDPVVKYRAF-APPKHYKRIHPDKTIQAPRWLSTDL
Saccharomyces cerevisiae LEU4 MYKESIALAEHAASRASRVIPPVYKLAYKNHLKDPSSKYKPF-NAPKLSNRKWPONRITRAPRWLSTDL
Kluyveromyces lactis LEU4 MIFRNTVYRLAQAG----KKAIPPVYKLAYKNHLKDPSTKYRPF-PQINLENRQWPSKTIITKAPRWLSTDL
Nakazawaea wickerhamii II MFKRTVYNLAKYG-----KSFEPTYKSNHLKDPSSKYIPY-PGVNMASTRDPSSKITKAPRWLSTDL
Peterozyma xilosa II MFKKSGIVFSKAA-----KSFEPTFKNHLRDPSSKYKAY-PGVQMESRDWPSSKITKAPRWLSTDL
Pachysolen tannophilus II MFKKTYYAFQKSAILK-----EGVVTAYKNLLKDPSSLKYKSS-PKINLSNRQWPSKTIERRAPRWLSTDL
Kluyveromyces lactis LEU4Bis MPFYKDPSSVYKYPFVSNVYKLQDRKWPSSKTLNKAAPRWLSTDL
Nakazawaea wickerhamii I MYNHLKDPSSVYKNKF-QPTNLPNRQWPNKTLDPKPRWLSTDL
Peterozyma xilosa I MYNHLKETSLKYTKF-QPLDLPNRQWPSKSIDKPPRWLSTDL
Pachysolen tannophilus I MGNHLKDPSSVYKYPF-TPIDLPNREWPSSKTLDPKPRWLSTDL

Supplementary Figure S3. Amino-terminus of α -IPMS proteins from representative species. The sequences shown were extracted from a MultAlin alignment (<http://multalin.toulouse.inra.fr/multalin/>) (Corpet, 1988). The red arrow indicates the highly conserved, alternative initiation methionine of the ScLeu4 protein, which leads to a cytosolic localized isoform (Beltzer et al., 1986; Beltzer et al., 1988). Species in red correspond to species of the CUG-Ala Clade (Shen et al., 2018), the presumed root of the α -IPMS gene duplication in the Saccharomycotina (see **Supplementary Data, Supplementary Figure S2** and text). Analysis of these sequences with DeepLoc-1.0 scored the proteins of the CUG-Ala Clade labelled I as cytosolic, and those labelled as II as mitochondrial. The coloring of residues is automatically set by the program and follows the standard Multalin default convention: residues in red, high consensus value; residues in blue, low consensus value; residues in black, no consensus at that position.