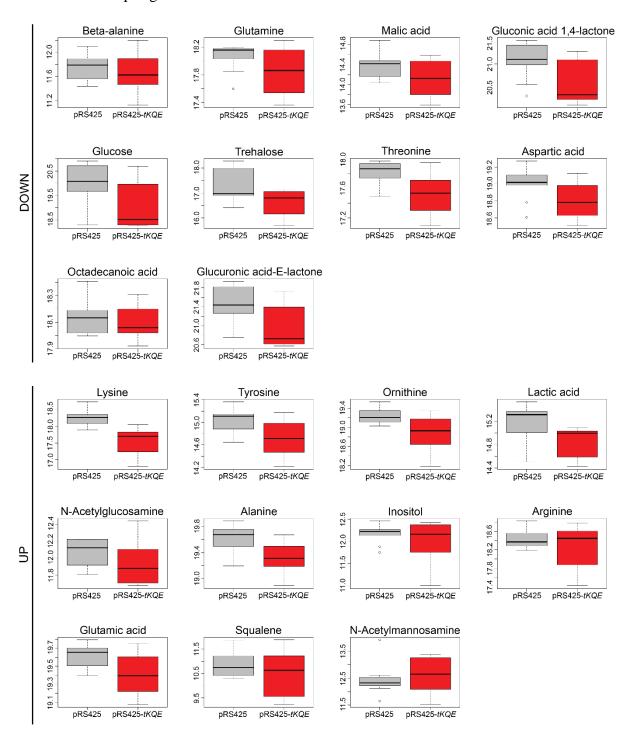
## Loss of ncm<sup>5</sup> and mcm<sup>5</sup> wobble uridine side chains results in an altered metabolic profile

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Online Resource 9. Suppression analysis comparing the  $elp3\Delta$ -h.c.-empty and  $elp3\Delta$ -h.c.-tKQE strains grown at 34°C. The  $elp3\Delta$  strain containing either an empty high copy pRS425 vector or a pRS425 high copy vector carrying the tRNA genes tK(UUU), tQ(UUG) and tE(UUC) (pRS425-tKQE) was grown logarithmically to an OD<sub>600</sub> of ~0.5 and harvested (see material and methods). Metabolites were extracted and then quantified using GC-TOF-MS. Values obtained were normalized and log2-transformed, and metabolic alterations were analyzed using PLS-DA. Metabolites shown have a VIP score >1 in the PLS-DA model comparing the  $elp3\Delta$ -1.c.-empty and  $elp3\Delta$ -1.c.-ELP3 strains grown at 34°C. Metabolites were classified as either UP (increased) or DOWN (decreased) in the  $elp3\Delta$ -1.c.-empty strain when compared with the  $elp3\Delta$ -1.c.-ELP3 strain. Boxplots were generated using R software with the Y-axis displaying the relative intensity in log2-scale.