Supplementary Material

# Supplementary Figure Captions

Figure S1: Growth kinetics of ~ 200 *Saccharomyces* strains on solid YP medium with 2 % (w/v) maltotriose (Mtt) at 30 oC generated with the PHENOS pipeline (Barton et al., 2018) represented as boxplots of (**A**) growth rate [h-1] (maximum slope), (**B**) adaptation time [h] (lag time) and (**C**) biomass production (maximum OD600 values). It has to be highlighted that the maltotriose-negative *S. eubayanus* strain CBS 12357T exhibited growth in the conditions tested. Data points are split based on species/domesticated hybrids designations. The species/domesticated hybrids are labelled as follows: *S. pastorianus* (▲), (*S. bayanus*) (■), *S. cerevisiae* (◉), *S. eubayanus* (●), *S. mikatae* (▼), *S. kudriavzevii* (♦), *S. uvarum* (■), *S. paradoxus* (⬣), *S. arboricola* (★). Values are presented as mean from quadruplicate technical replicates. Raw data is provided in Tables S4 and S5.

Figure S2: Growth characteristics of 80 *Saccharomyces* strains generated in the PHENOS pipeline (Barton et al., 2018) on synthetic medium with 2 % (w/v) maltotriose (Mtt) at 25 oC represented as boxplots of (**A**) growth rate [h-1] (maximum slope), (**C**) adaptation time [h] (lag time) and (**D**) biomass production (maximum OD600 values). Data points are split based on species designations. Data points of maltotriose-positive candidates based on growth rate values larger than the value of the maltotriose-negative (Mtt-) strain *S. eubayanus* strain CBS 12357T are highlighted with a larger symbol with full color and separated by a red dash line in panel (A). (**B**) Growth performance on maltotriose ranked by growth rate [h-1] (maximum slope values). The value for the strain *S. eubayanus* strain CBS 12357T (Mtt-) was set to 1 and the performances of all other strains were shown as fold-change in comparison. The species/domesticated hybrids are labelled as follows: *S. pastorianus* (▲), *S. cerevisiae* (◉), *S. eubayanus* (●), *S. mikatae* (▼), *S. kudriavzevii* (♦), *S. uvarum* (■), *S. paradoxus* (⬣), *S. arboricola* (★), *S. jurei* (★). Values are presented as a mean of quadruplicate technical replicates while valued for control strains maltotriose-positive (Mtt+) *S. pastorianus* CBS 1513 and *S. eubayanus* strain CBS 12357T (Mtt-) are shown as mean of twenty-four replicates. Raw data is provided in Table S6.

# Supplementary Table caption for Table S9

Table S9: Quantitative data for the aroma production of the generated *de novo* hybrids and the corresponding parental strains: NG92 (*S. eubayanus* CBS 12357T x *S. jurei* D5095T/*Se* x *Sj*), NG101 (*S. eubayanus* CBS 12357T x *S. mikatae* NBRC 10997/*Se* x *Sm*), *S. eubayanus* CBS 12357T, *S. jurei* D5095T and *S. mikatae* NBRC 10997 including the strain *S. pastorianus* CBS 1513 as a typical lager beer reference. The aroma compounds are categorized by acetate esters, ethyl esters, alcohols, and acids (medium-chain fatty acids (MCFA). The concentration differences per category are highlighted with increasing length of blue, green, orange, and yellow bars respectively. Flavor thresholds in beer values (shown in brackets) were obtained from Meilgaard, 1982 (a); Meilgaard, 1975a (b); (Meilgaard, 1975b (c); Harrison, 1970 (d). Corresponding aromas/flavors for each volatile compound tested were obtained from The Good Scent Company Information System, 2022 (e); Swiegers et al. (2005) (f); PubChem, 2022 (g); Dunlevy et al. (2009) (h); Blanco et al. (2016) (i). The calculations are based on aroma compound concentrations [mg L-1] from triplicate biological fermentations including values below and above the sensitivity of the GC/MS analysis. Statistical analysis shows significant difference for each aroma compound tested with P value < 0.05 (Table S11).

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