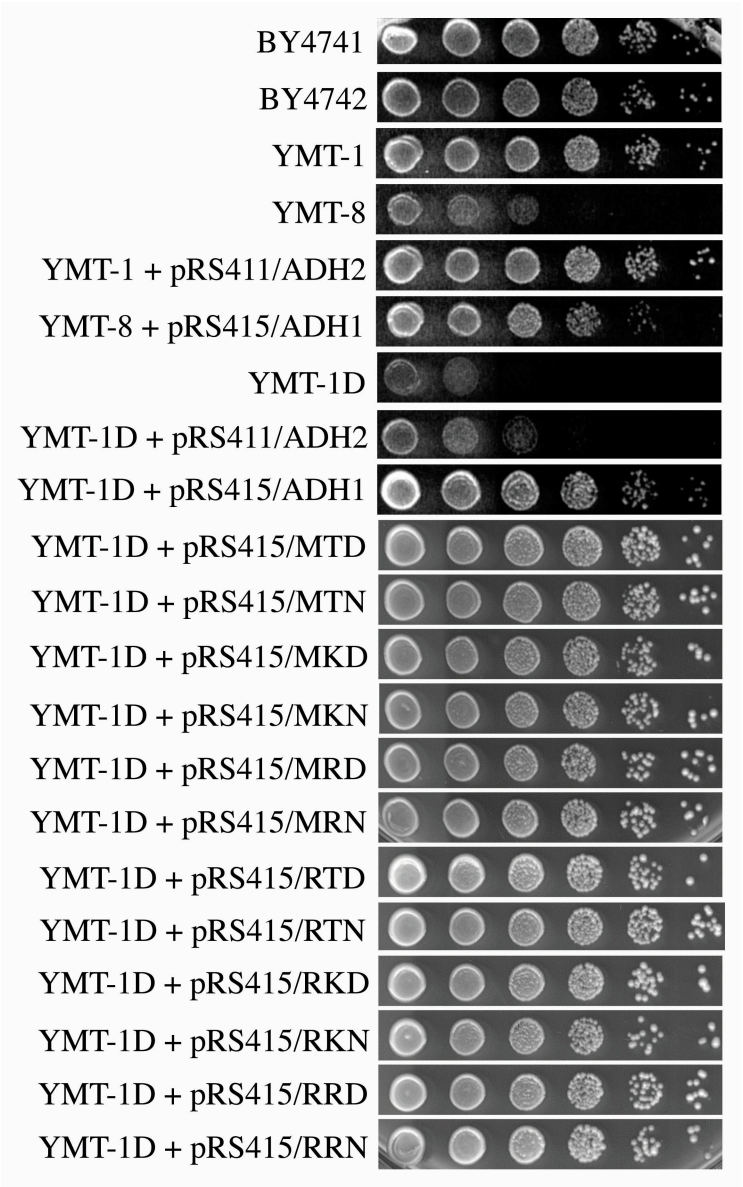
BY4741
BY4742
YMT-1
YMT-8
YMT-1 + pRS411/ADH2
YMT-8 + pRS415/ADH1
YMT-1D
YMT-1D + pRS411/ADH2
YMT-1D + pRS415/ADH1
YMT-1D + pRS415/MTD
YMT-1D + pRS415/MTN
YMT-1D + pRS415/MKD
YMT-1D + pRS415/MKN
YMT-1D + pRS415/MRD
YMT-1D + pRS415/MRN
YMT-1D + pRS415/RTD
YMT-1D + pRS415/RTN
YMT-1D + pRS415/RKD
YMT-1D + pRS415/RKN
YMT-1D + pRS415/RRD
YMT-1D + pRS415/RRN

Supplementary Figure 1 shows the complementation of Adh 1/2 double minus with ancestral proteins

Figure 1: Pin stamp analysis by plasmid rescue. The *adh1* (YMT-8), *adh2* (YMT-1), and *adh1/adh2* (YMT-1D) deletion strains were rescued with either *Saccharomyces cerevisiae* ADH1, ADH2, or anyone of the ADH_As. Yeast were serially diluted 10 fold from left to right on 2% glucose minimal medium plates with supplements. The double deletion mutant has a phenotype exhibiting extremely slowly on glucose-containing medium, with a doubling time of over 2000 minutes.
Each of the ancestral sequences produced functional enzymes capable of rescuing the double deletion phenotype, allowing the strain to grow again on glucose as the sole carbon source. Given that a total 15 amino acid changes were made to each enzyme (12 static and 3 combinatorial), this observation reveals the robustness of the reconstruction to create active enzymes. However, this does not allow any remarks as to the catalytic or kinetic activity of the ancestor, as expression of any adh from the Adh1 promoter could alleviate this growth phenotype.