

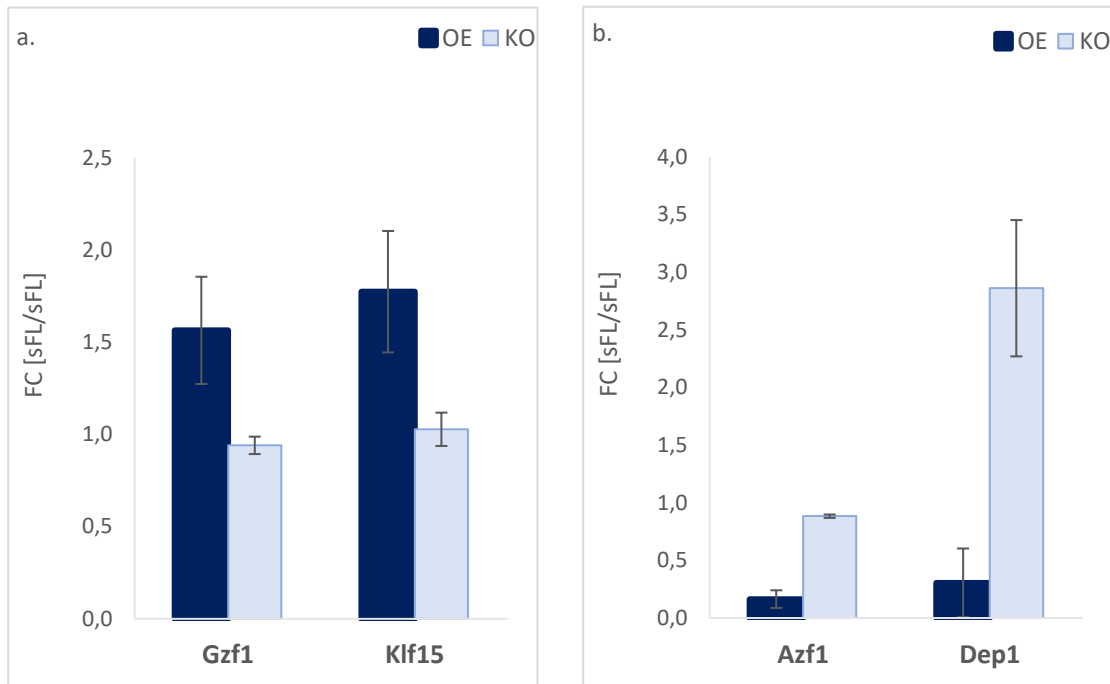
Figure S1.

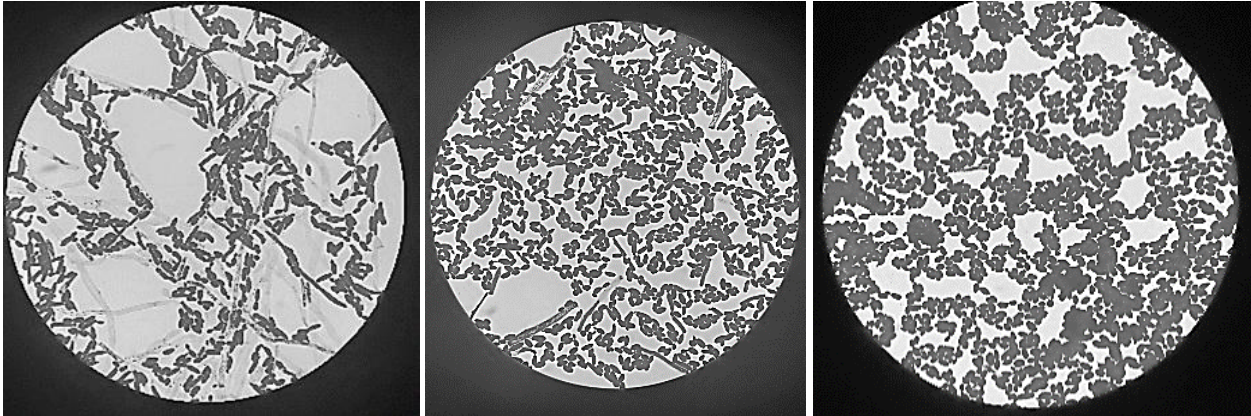
Figure S1. Recombinant protein production by *Y. lipolytica* strains engineered in genes encoding selected Transcription Factors by their overexpression (OE) or deletion (KO). The engineered genes were: Gzf1 (YALIOD20482g), Klf1 (YALIOD05041g), Azf1 (YALIOA16841g), Dep1 (YALIOF05896g). The OE strains were constructed as described in (Leplat et al. 2015). The KO strains were constructed using a CRISPR-Cas9 system described in (Larroude et al. 2020). The constructed strains were cultured for 48 h in the medium composed as follows [g L⁻¹]: yeast nitrogen base, 5.1 (Sigma-Aldrich); ammonium sulfate, 15, (POCH); glucose, 25 (POCH); buffered with 0.2 M maleic acid at pH 5.0. The results are presented as Fold Change values of specific Fluorescence from RedStarII [FL / OD600] of the TF-engineered strain over sFL of the control strain Y2810 (expressing RedStarII) [sFL/sFL]. sFL – fluorescence intensity normalized per biomass. FC – ratio between sFL for the TF-engineered strain over the control strain. Error bars show $\pm$ SD from three biological replicates.

Figure S2.

Azf1

Mhy1

Msn4



Y2810

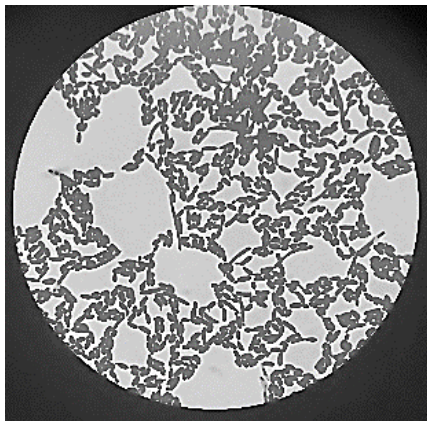


Figure S2. Microscopic images of *Y. lipolytica* strains overexpressing one of the selected Transcription Factors Azf1 (YALI0A16841g), Mhy1 (YALI0B21582g), Msn4 (YALI0C13750g), and the control strain. The yeasts' biomass was prepared by their culturing over 24 h in liquid YPD medium ([g L⁻¹]: yeast extract, 10 (Biomaxima); peptone, 15, (Biomaxima); glucose, 20 (POCH). Biomass was briefly centrifuged, washed in sterile saline solution, and placed on a microscope slide to prepare fixed preparation stained with crystal violet. The images were taken under 1000x magnification using a Zeiss Primo Star microscope.

Figure S3.

		growth	Total r-Prot	Normalized r-Prot
TF036 (YALIOD20460g)	pH	0,3	1,1	
	Temp	1,1		
	OA	14,6	12,4	
	C	3,5		1,6
	N	3,3	1,8	1,1
Jmc2 (YALIOB14443g)	pH			
	Temp		1,0	1,3
	OA	10,6	2,9	2,5
	C	1,3	0,5	1,0
	N	0,7		1,4
TF011 (YALIOB20944g)	pH			
	Temp		1,5	1,5
	OA	7,3	5,4	
	C	0,8	0,8	
	N	0,4	1,3	
Dal81 (YALIOD02783g)	pH	0,6		0,7
	Temp	3,0	2,0	
	OA	2,7	0,6	0,5
	C		0,7	
	N			0,4
Hoy1 (YALIOA18469g)	pH	11,1	2,1	3,7
	Temp	3,3	1,1	0,5
	OA	2,7	1,0	
	C	1,1	1,5	
	N		4,7	7,2

Figure S3. Factor's contribution rankings in terms of growth, r-Prots synthesis, and normalized measure of r-Prots synthesis based on mathematical models developed using FC values readouts, for TFs: TF036 (YALIOD20460g), Jmc2 (YALIOB14443g), TF011 (YALIOB20944g), Dal81 (YALIOD02783g), and Hoy1 (YALIOA18469g). Ranking tables are color-coded according to a convention presented in **Figure 1**. Percentage contribution values discussed in the manuscript are bolded.