

Supplementary Information:

Table S1: Gene copy numbers of four GOIs in three engineered *Pichia pastoris* strains after three consecutive batch cultivations. Clone 1, 2 and 3 contain 3/3/1/1, 3/3/3/1 and 2/2/2/2 copies of GOI 1-4, respectively. GCN were normalized to the first sample point. Gene copy number analysis was done in triplicates or quadruplicates as described before [40]. Standard deviations were below 7% for all measurements.

	batch 1				batch 2				batch 3			
	GOI1	GOI2	GOI3	GOI4	GOI1	GOI2	GOI3	GOI4	GOI1	GOI2	GOI3	GOI4
clone 1	1	1	1	1	1.0	1.2	1.0	1.0	0.9	1.3	1.0	1.1
clone 2	1	1	1	1	0.9	1.0	0.8	1.0	1.0	1.3	0.9	0.8
clone 3	1	1	1	1	0.9	1.0	0.9	0.9	1.2	1.4	1.1	1.2

Table S2: *P. pastoris* deep-well screening conditions representing specific bioreactor cultivation phases

Condition	ID	Start-OD ₆₀₀	Cultivation substrate	Cultivation time [h]	Harvest-OD ₆₀₀	Harvest-μ [h ⁻¹]	Represented bioreactor cultivation phase
Excess glycerol	G	0.1	2% glycerol	15-20 h	3-8	0.23	glycerol batch
Excess glucose	D	0.1	2% glucose	15-20 h	3-8	0.23	glucose batch
Limiting glucose	X	1	12 mm glucose feed bead 0.5% and 1% methanol (at start and after 16h)	15-20 h	8-12	0.04 (av. 0.14)	glucose fed batch
Methanol feed	M	4		16 + 6 h	8-12	0.10	methanol shot/feed

Table S3: Selection markers. Antibiotics, resistance genes and working concentrations for *P. pastoris* and *E. coli* are listed.

Antibiotic	Resistance	working concentration <i>P. pastoris</i> [μg/mL]	working concentration <i>E. coli</i> [μg/mL]
Ampicillin	ampR	100	100
Kanamycin	kanMX	50	50
Geneticin (G418)	kanMX	500	500
Zeocin	zeoR (Sh ble)	25	25
Hygromycin	hphMX	200	50
Nourseothricin	natMX	100	50

Table S4: Sequences of gRNA's and verification primers for Indel mutations using CRSIPR/Cas9

target	gRNA_1	gRNA_2	Sequencing_primer_fw	Sequencing_primer_rv
eGFP	GGGCGAGGAGCTGTTACCG	CGCCGGACACGCTGAACTTG	TTGCCCTTTCACTTGAC	CCTTAACTTGCCAAACCT
AOX1	CTAGGATATCAAACCTTCG	GACTGTTCTCAGTTCAAGT	TCTTGGAACCTAATATGACAAAAGC	TTCTTCGCAGGCTTGGCTT
DAS1	AAGCATGACAAGTTGTAAAC	TGGAGAATAATCGAACAAAA	TTCATGTCGCTAAGGCTTTCTT	TTTCAGGTAGCGTCAGATTCGG