Supplemental Material for Publication

Table S1. Single nucleotide variations (SNV's) and gene duplication identified in MG genome.

Single nucleotide variations (SNV's)			
Systematic name	**Name**	**Type**	**Amino acid change**
YER001W	***MNN1***	**MisSense**	**L322F**
YBR163W	***EXO5***	**MisSense**	**A195E**
YML007W	***YAP1***	**MisSense**	**Q171K**
YMR115W	***MGR3***	**MisSense**	**N89D**
YLR233C	***EST1***	**MisSense**	**A275V**
YLR249W	***YEF3***	**MisSense**	**A99T**
YKL073W	***LHS1***	**MisSense**	**E287K**
YJL019W	***MPS3***	**MisSense**	**Q201E**
YPL097W	***MSY1***	**MisSense**	**A311V**
YNR075W	***COS10***	**MisSense**	**V70L**
YLL040C	***VPS13***	**Sense**	**I1944I**
Gene duplications			
Systematic name	**Name**	**Function**	
Chromosome III			
YCR019w	***MAK32***	Protein necessary for structural stability of L-A double-stranded RNA-containing particles (1)	
YCR020C	***PET18***	Protein of unknown function	
YCR020C-A	***MAK31***	Non-catalytic subunit of N-terminal acetyltransferase of the NatC type	
YCR021C	***HSP30***	Hydrophobic plasma membrane localized, stress-responsive protein that negatively regulates the H(+)-ATPase Pma1p; induced by heat shock, ethanol treatment, weak organic acid, glucose limitation, and entry into stationary phase	

YCR022C		Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; YCR022C is not an essential gene
YCR023C		Vacuolar membrane protein of unknown function; member of the multidrug resistance family; YCR023C is not an essential gene
YCR024C	***SLM5***	Mitochondrial asparaginyl-tRNA synthetase
YCR024C-A	***PMP1***	Regulatory subunit for the plasma membrane H(+)-ATPase Pma1p
YCR024C-B		Putative protein of unknown function
YCR025C		Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; YCR025C is not an essential gene
YCR026C	***NPP1***	Nucleotide pyrophosphatase/phosphodiesterase; mediates extracellular nucleotide phosphate hydrolysis along with Npp2p and Pho5p; activity and expression enhanced during conditions of phosphate starvation; *NPP1* has a paralog*, NPP2*, that arose from the whole genome duplication
YCR027C	***RHB1***	Putative Rheb-related GTPase involved in regulating canavanine resistance and arginine uptake; member of the Ras superfamily of G-proteins
Chromosome V		
YER093C-A	***AIM11***	Protein of unknown function
YER094C	***PUP3***	Beta 3 subunit of the 20S proteasome involved in ubiquitin-dependent catabolism; human homolog is subunit C10
YER095W	***RAD51***	Strand exchange protein, forms a helical filament with DNA that searches for homology; involved in the recombinational repair of double-strand breaks in DNA during vegetative growth and meiosis; homolog of Dmc1p and bacterial RecA protein
YER096W	***SHC1***	Sporulation-specific activator of Chs3p (chitin synthase III); required for the synthesis of the chitosan layer of ascospores; transcriptionally induced at alkaline pH; *SHC1* has a paralog, *SKT5*, that arose from the whole genome duplication
YER097W		Dubious open reading frame unlikely to encode a functional protein, based on available experimental and comparative sequence data
YER098W	***UBP9***	Ubiquitin-specific protease that cleaves ubiquitin-protein fusions; UBP9 has a paralog, UBP13, that arose from the whole genome duplication
YER099C	***PRS2***	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, synthesizes PRPP, which is required for nucleotide, histidine, and tryptophan biosynthesis; one of five related enzymes, which are active as heteromultimeric complexes; *PRS2* has a paralog*, PRS4*, that arose from the whole genome duplication
YER100W	***UBC6***	Ubiquitin-conjugating enzyme involved in ER-associated protein degradation; located at the cytosolic side of the ER membrane; tail region contains a transmembrane segment at the C-terminus; substrate of the ubiquitin-proteasome pathway
YER101C	***AST2***	Lipid raft associated protein; overexpression restores Pma1p localization to lipid rafts which is required for targeting of Pma1p to the plasma membrane; sometimes classified in the medium-chain dehydrogenase/reductases (MDRs) superfamily; *AST2* has a paralog, *AST1*, that arose from the whole genome duplication
YER102W	***RPS8B***	Protein component of the small (40S) ribosomal subunit; homologous to mammalian ribosomal protein S8, no bacterial homolog; *RPS8B* has a paralog, *RPS8A*, that arose from the whole genome duplication
YER103W	***SSA4***	Heat shock protein that is highly induced upon stress; plays a role in SRP-dependent cotranslational protein-membrane targeting and translocation; member of the HSP70 family; cytoplasmic protein that concentrates in nuclei upon starvation; *SSA4* has a paralog, *SSA3,* that arose from the whole genome duplication
YER104W	***RTT105***	Protein with a role in regulation of Ty1 transposition (1)

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Table S2. Differentially expressed MG's genes under aerobic conditions.

Up-regulated		
Systematic name	Name	Fold-change
YCR019W	*MAK32*	2.1
YCR020C	*PET18*	2.1
YCR020C-A	*MAK31*	2.2
YCR020W-B	*HTL1*	2.3
YCR021C	*HSP30*	2.0
YCR026C	*NPP1*	2.1
YDR046C	*BAP3*	2.2
YER094C	*PUP3*	2.2
YER095W	*RAD51*	2.3
YER099C	*PRS2*	2.3
YER100W	*UBC6*	2.1
YER101C	*AST2*	2.0
YER104W	*RTT105*	2.8
Down-regulated		
Systematic name	Name	Fold-change
YLR159W	---	-4.3
YCL018W	*LEU2*	-3.6
YLR155C	*ASP3-1*	-2.6
YOR383C	*FIT3*	-2.4

9 **Table S3. Strains used in this study.**

Strain name	Relevant genotype	Reference
CEN.PK102-12A	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2*	(1-3)
CEN.PK113-7D	MAT*a MAL2-8c SUC2*	(1-3)
IMX076	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5*	This study
IMX080	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2*	This study
IMX096	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX*	This study
IMX098	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr*	This study
IMI175	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr, gpm2::KlURA3*	This study
IMX126	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KLLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3::KlURA3*	This study
IMX135	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KLLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3, eno1::KlURA3*	This study
IMX142	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3, eno1, pyk2::KlURA3*	This study
IMX151	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3, eno1, pyk2, pdc5::KlURA3*	This study

IMX158	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3, eno1, pyk2, pdc5, pdc6::KlURA3*	This study
IMX163	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3, eno1, pyk2, pdc5, pdc6, adh2::KlURA3*	This study
IMX175	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3, eno1, pyk2, pdc5, pdc6, adh2, adh5::KlURA3*	This study
IMX208	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KanMX, tdh2::Hygr, gpm2, gpm3, eno1, pyk2, pdc5, pdc6, adh2, adh5, adh4::KlURA3*	This study
IMX343	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KlURA3, tdh2::Hygr, gpm2, gpm3, eno1, pyk2, pdc5, pdc6, adh2, adh5, adh4*	This study
IMX346	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KlURA3, tdh2::amdSYM, gpm2, gpm3, eno1, pyk2, pdc5, pdc6, adh2, adh5, adh4*	This study
IMX370	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1, tdh2, gpm2, gpm3, eno1, pyk2, pdc5, pdc6, adh2, adh5, adh4*	This study
IMX372 - Minimal Glycolysis (MG)	MAT*a ura3-52 his3-1 leu2-3,112 MAL2-8c SUC2 glk1::SpHis5, hxk1::KlLEU2, tdh1::KlURA3, tdh2, gpm2, gpm3, eno1, pyk2, pdc5, pdc6, adh2, adh5, adh4*	This study

12 **Table S4. Primers used in this study.**

Name	Sequence 5'-> 3'	Targeted gene/plasmid
Deletion cassette construction		
glk1dFW/1712	**AAACCACAACACCACCACTAATACAACTCTATCATACAC AAGATG**CCGCCAAGCGAATTGAAGGA*CAGCTGAAGCTTC GTACGC*	*GLK1*/pUG27
glk1dRV/1713	**GTACGGTGGGATACGTACACAAACCAAAAAAATGTAAAA AGATCA**CCGTGCGTAGAATGAAGAAC*GCATAGGCCACTA GTGGATCTG*	*GLK1*/pUG27
hxk1dFW/1710	**AAACTCACCCAAACAACTCAATTAGAATACTGAAAAAAT AAGATG**ATGACAAGAGGGTCGAACTC*CAGCTGAAGCTTC GTACGC*	*HXK1*/pUG73
hxk1dRV/1711	**AGGGAGGGAAAAACACATTTATATTTCATTACATTTTTT TCATTA**GCCTAAGTCGTAATTGAGTC*GCATAGGCCACTA GTGGATCTG*	*HXK1*/pUG73
TDH1 FW deletion/1800	**ACACAAAAAACAGTACTTCACTAAATTTACACACAAAAC AAAATG**TATGCCAGCTACCTAGTGCG*CAGCTGAAGCTTC GTACGC*	*TDH1*/pUG6 or pDS2 or pDS3
TDH1 RV deletion/1801	**ATATTCAAAAAAAAATCATTATCCTCATCAAGATTGCTT TATTTA**GGCCATATCACATTAACTGC*GCATAGGCCACTA GTGGATCTG*	*TDH1*/pUG6 or pDS2 or pDS3
TDH2 FW deletion/1802	**TTAGTTTCAAATTAAATTCATCACACAAACAAACAAAAC AAAATG**CCGCTTCATCAAGACTGTTA*CAGCTGAAGCTTC GTACGC*	*TDH2*/pUG-hphN or pDS2 or pDS3
TDH2 Rv deletion/1803	**ATAATAAAAACTAAATCATTAAAGTAACTTAAGGAGTTA AATTTA**TAGCAGCCGTGCATACGCAT*GCATAGGCCACTA GTGGATCTG*	*TDH2*/pUG-hphN or pDS2 or pDS3
GPM2DcsmlFW/2478	**TTAAACCCAAGAATACATAAAAAAAATATAGATATATTA ACTTAGTAAACAATG***CAGCTGAAGCTTCGTACGC*	*GPM2*/pUG72
GPM2DcsmlRV/2479	**TGGTTTTCATTATACTTCGGAAAATACACAATTATATTA TATACTTACCCCCCTTA**ATTGAACAACCTCGTATTTGGA TGTGTGTCTCGAAACACT*GCATAGGCCACTAGTGGATCT G*	*GPM2*/pUG72
GPM3DcsmlFW/2480	**ATTGAGAAATAGTGCAAAAAGATCTACTAATAACGAATA GTTATG**AACATCGAGCTGAGCATCAACACCTTTGACGAG CTGTTCACC*TCGAGAGCTCGTTTTATTTAGGTTC*	*GPM3*/pUG72
GPM3DcsmlRV/2481	**AGGAAACCATGAAAAAAATGGCGCTAATTTTTTATTTTT AAAAACTATTCA**AGAGACTTTTATTGTAAATCCTCGATT GCAGGTTGTTTTA*GCATAGGCCACTAGTGGATCTG*	*GPM3*/pUG72
ENO1DUFW/2411	**AAACCAAGCAACTGCTTATCAACACACAAACACTAAATC AAAATG***TCGAGAGCTCGTTTTATTTAGGTTC*	*ENO1*/pUG72
ENO1DURV/2412	**AAAAAAACGTGTTTTTTGGACTAGAAGGCTTAATCAAAA GCTTTA**TTTATGAAAAATAGCTAGAAGGAATAAGGGATT ACAAGAGAGATGTTACA*AGAGATCCCAATACAACAGATC AC*	*ENO1*/pUG72
PYK2smlFW/7420	**CTATATTTTACTTTCATCCTCTACGTCCATTGTAAGATT ACAACAAAAGCACTATCGATG***TCGAGAGCTCGTTTTATT TAGGTTC*	*PYK2*/pUG72
PYK2smlRV/7421	**GACAATTAAATAAAATTAAGTAAAAAAAAATAAGGACTTT AATTTT**TACTATTTCACCGCTCTGCTTCAAAATGTTTTA TGTTCTTTGTTTTCTTT*AGAGATCCCAATACAACAGATC AC*	*PYK2*/pUG72
PDC5DCFW-1/2802	**TTACACTTATTTCACATAATCAATCTCAAAGAGAACAAC ACAATACAATAACAAGAAGAACAAAAT***TCGAGAGCTCGT TTTATTTAGGTTC*	*PDC5*/pUG72
PDC5DCRV-1/2760	**GTAAAAAAATACACAAACGTTGAATCATGAGTTTTATGT TAATTAGCTTA**TAAGAAAGAGAGGAAAGGACTTACTACA GTATATTGATCG*AGAGATCCCAATACAACAGATCAC*	*PDC5*/pUG72
pdc6DcRV/2459	**TATTTGCAACAATAATTCGTTTTTGAGTACACTACTAAT GGCTTA**TACTGTATATAAAAGAGGACTGCAATAGCACAA GATTAAG*GCATAGGCCACTAGTGGATCTG*	*PDC6*/pUG72

pdc6DcFW/2458	**TAAAAAACCCAAGTAATATAGCAAAAACATATTGCCAAC AAAATG***CAGCTGAAGCTTCGTACGC*	*PDC6*/pUG72
ADH2DCFW/2804	**ATCAAGCTACAAAAAGCATACAATCAACTATCAACTATT AACTATATCGTAATACACAAT***TCGAGAGCTCGTTTTATT TAGGTTC*	*ADH2*/pUG72
ADH2DCRV/2761	**ATGCTTGATAATGAAAACTATAAATCGTAAAGACATAAG AGATCCGCTTA**ATTCTATTTACCAAGAAGAAACAAGAAG TGATAAAAACAA*AGAGATCCCAATACAACAGATCAC*	*ADH2*/pUG72
ADH5DCFW/2808	**CTGATTGGAAGATACCTAAGAAAATTATTTAACTACATA TCTACAAAATCAAAGCATCAT***TCGAGAGCTCGTTTTATT TAGGTTC*	*ADH5*/pUG72
ADH5DCRV/2763	**GCTTATATAAAAAGTAAAAATATATTCATCAAATTCGTT ACAAAAGATCA**AGACATTGTGAGACAGTAAAGCAGTAGT TTGCGCTAGAAA*AGAGATCCCAATACAACAGATCAC*	*ADH5*/pUG72
ADH4DCFW/2806	**AAAAAAAAAGAACTAGTTTTTAGTTCGCGCATCACGAGG TACGTGTTTAATATGTCAGAT***TCGAGAGCTCGTTTTATT TAGGTTC*	*ADH4*/pUG72
ADH4DCRV/2762	**AAATAAGGCACACGCATAATTGACGTTTATGAGTTCGTT CGATTTTTTTA**TTTTCTATAGCTAATCCACTGCGGTGAT ACTACAGCCATC*AGAGATCCCAATACAACAGATCAC*	*ADH4*/pUG72

Deletion confirmation

GLK1FW2/1524	ATCAGTGCCCAACTCAGCTTCC	*GLK1*
GLK1RV2/1525	AACCAAAGGCCCGTTTCCGATG	*GLK1*
KanB r/114	CGACCAGCATTCACATACGA	*TEF2*
KanA f/113	CTTGACAGTCTTGACGTGCG	*TEF2*
HXK1FW/1716	GACCGCAAAAAAAACATAAGGG	*HXK1*
HXK1RV/1717	CCGTTCCTTCATCTTGTATTCTTC	*HXK1*
iLEU2RV/1721	GTTAGTGTCAGGTAGGGAAGC	*LEU2*
iLEU2FW/1720	CAATTCAGCGCAGTCACG	*LEU2*
Ctdh1FW/1989	CCACGTGCAGAACAACATAG	*TDH1*
Ctdh1RV/1990	ATAGTCACATATTGTGGGTATGTG	*TDH1*
KanA/9	CGCACGTCAAGACTGTCAAG	*TEF2*
Kan B/10	TCGTATGTGAATGCTGGTCG	*TEF2*
TDH2CFW/2350	TCAAGTTCCCATTTGGCAATC	*TDH2*
TDH2RV/2351	TGGGTCGGCCTGTTGTTTC	*TDH2*
hygroFW/1993	GGACGCTCGAAGGCTTTAAC	*Nph*
gpm2confFW/2482	GTTATCACCCCACGACGAAG	*GPM2*
gpm2confRV/2483	TCTGCATTCAGGAATGTTCTTATAAATATC	*GPM2*

UB/2300	GAAATGCTGGATGGGAAGCG	*URA3*
UA/2299	GGCCCAATCACAACCACATC	*URA3*
gpm3confFW/2484	TGCGAGATTTCATTGACAAGTTCG	*GPM3*
gmp3confRV/2485	TACGAGTAGATAAGATGGCTTATGC	*GPM3*
ENO1ConFW/2305	TTCTGGCACACATGATCTCC	*ENO1*
ENO1ConRV/2306	ACATGGGTGACCAAAAGAGC	*ENO1*
PYK2ConFW/2307	CGCAGTTTGCGAACATTACC	*PYK2*
PYK2ConRV/2308	TTTATTTAGCGACGCAGCATAG	*PYK2*
pdc5DconfFW/2805	ATGAGACTTGAATAATGCAGCG	*PDC5*
pdc5DconfRV/2807	GGTTAAAGATCACACCACCC	*PDC5*
pdc6confFW/2460	AGAGACGCGCAGTACGTAAC	*PDC6*
pdc6confRV/2461	TATGCAGATCGGCTGTGGC	*PDC6*
adh2DconfFW/2765	GAACACCGGGCATCTCCAAC	*ADH2*
adh2DconfRV/2766	CGAACACTGCTGAAGCTACC	*ADH2*
adh5DconfFW/2769	TTCTCCTTTCGCGGAAGGATG	*ADH5*
adh5DconfRV/2770	CCAAATGTCCACCGGTTCTC	*ADH5*
adh4DconfFW/2767	TGTGTTCAGAAGGATCCCCG	*ADH4*
adh4DconfRV/2768	AAGGCACACGCATAATTGAC	*ADH4*

Bold: Sequence for targeted integration

Underlined: Sequence for seamless marker removal

Italic: Sequence for plasmid binding

Table S5. Plasmids used in this study.

Plasmid	Marker	Reference
pUG27	*Sphis5*	(4)
pUG73	*KlLEU2*	(4)
pUG72	*KlURA3*	(4)
pUG6	*KanMX*	(5)
pUG-hphNT1	$AgTEF2_{pr}$-*hphNT1*-$AgTEF2_{ter}$	(6)
pDS2	*amdSYM*	(7)
pDS3	*KlURA3*	(7)

Table S6. Experimental conditions used for expression data of glycolytic genes of *S. cerevisiae* according to (8). The transcript data are searchable at Genome Expression Omnibus (www.ncbi.nlm.nih.gov/geo) under the accession number GSE11452.

Experiment No.	Conditions
1	Aerobic glucose limited 1
2	Aerobic glucose limited 2
3	Aerobic glucose limited 3
4	Aerobic nitrogen limited 1
5	Aerobic nitrogen limited 3
6	Aerobic phosphorus limited 1
7	Aerobic phosphorus limited 2
8	Aerobic phosphorus limited 3
9	Aerobic sulfur limited 1
10	Aerobic sulfur limited 2
11	Aerobic sulfur limited 3
12	Anaerobic carbon limited 1
13	Anaerobic nitrogen limited 1
14	Anaerobic nitrogen limited 2
15	Anaerobic nitrogen limited 3
16	Anaerobic phosphorus limited 1
17	Anaerobic phosphorus limited 2
18	Anaerobic phosphorus limited 3
19	Anaerobic sulfur limited 1
20	Anaerobic sulfur limited 2
21	Anaerobic sulfur limited 3
22	Anaerobic carbon limited 4
23	Anaerobic carbon limited 5
24	Anaerobic carbon limited 6
25	Aerobic glucose limited 4
26	Aerobic glucose limited 5
27	Aerobic glucose limited 6
28	C-lim Anaerobic reference (pH 5) #1
29	C-lim Anaerobic reference (pH 5) #2
30	C-lim Anaerobic reference (pH 5) #3
31	C-lim Anaerobic Acetate 1
32	C-lim Anaerobic Acetate 2
33	C-lim Anaerobic Acetate 3
34	C-lim Anaerobic Benzoate 1
35	C-lim Anaerobic Benzoate 2
36	C-lim Anaerobic Benzoate 3
37	C-lim Anaerobic Propionate 1
38	C-lim Anaerobic Propionate 2
39	C-lim Anaerobic Propionate 3

40 C-lim Anaerbic Sorbate 1
41 C-lim Anaerobic Sorbate 2
42 C-lim Anaerobic Sorbate 3
43 12C Chemostat anaerobic C-lim 0.03h-1 (1)
44 12C C-im anaerobic chemostat 0.03h-1 (2)
45 12C anaerobic C-lim chemostat 0.03h-1 (3)
46 30C Anaerobic C-lim chemostat 0.03h-1 (1)
47 30C anaerobic C-lim chemostat 0.03h-1 (2)
48 30C anaerobic C-lim chemostat 0.03 h-1 (3)
49 12C anaerobic N-limitation chemostat 0.03h-1 (1)
50 12C anaerobic N-lim chemostat 0.03h-1 (2)
51 12C anaerobic N-lim chemostat 0.03h-1 (3)
52 30C anaerobic N-lim chemostat 0.03h-1 (1)
53 30C anaerobic N-lim chemostat 0.03h-1 (2)
54 30C anaerobic N-lim chemostat 0.03h-1 (3)
55 C-lim aerobic chemostat with ASN as N-source 1
56 C-lim aerobic chemostat with ASN as N-source 2
57 C-lim aerobic chemostat with ASN as N-source 3
58 C-lim aerobic chemostat with Proline as N-source 1
59 C-lim aerobic chemostat with proline as N-source 2
60 C-lim aerobic chemostat with proline as N-source 3
61 C-lim aerobic chemostat with leucine as N-source 1
62 C-lim aerobic chemostat with leucine as N-source 2
63 C-lim aerobic chemostat with leucine as N-source 3
64 C-lim aerobic chemostat with phenylalanine as N-source 2
65 C-lim aerobic chemostat with phenylalanine as N-source 3
66 C-lim aerobic chemostat with methionine as N-source 1
67 C-lim aerobic chemostat with methionine as N-source 2
68 C-lim aerobic chemostat with methionine as N-source 3
69 Zinc limited Aerobic chemostat culture -1
70 Zinc-limited aerobic chemostat culture-2
71 Zinc-limited Aerobic chemostat culture -3
72 Zinc-limited Anaerobic chemostat culture-1
73 Zinc-limited Anaerobic chemostat culture-2
74 Zinc-limited Anaerobic chemostat culture-3
75 Carbon-limited Aerobic chemostat culture -1
76 Carbon-limited Aerobic chemostat culture-2
77 Carbon-limited Aerobic chemostat culture-3
78 Nitrogen-limited Aerobic chemostat culture-1
79 Nitrogen-limited Aerobic chemostat culture-2
80 Nitrogen-limited Aerobic chemostat culture-3
81 Nitorgen-limited Anaerobic chemostat culture-1
82 Nitrogen-limited Anaerobic chemostat culture-3
83 Nitrogen-limited Anaerobic chemostat culture-2
84 CENPK113-7D glucose/ethanol limited chemostat -1
85 CENPK113-7D glucose/ethanol limited chemostat -2

86	CENPK-113-7D glucose/ethanol limited chemostat -3
87	CENPK113-7D glucose/ethanol limited chemostat -4
88	Ethanol limited chemostat D=0.1/h -1
89	Ethanol limited chemostat D= 0.1 /h -2
90	Ethanol limited chemostat D=0.1/h -3
91	Acetate limited chemostat culture D=0.1/h -1
92	Acetate limited chemostat culture D=0.1/h -2
93	Acetate limited chemostat D=0.1/h -3
94	Maltose limited chemostat culture D=0.1/h -3
95	Anaerobic glucose limited chemostat culture with 100%CO2
96	Anaerobic glucose limited chemostat culture with 100% CO2 D=0.1/h -2
97	Anaerobic glucose limited chemostat culture with 100% CO2 D=0.1/h -3
98	Aerobic glucose limited chemostat culture with 79% CO2 D=0.1/h -1
99	Aerobic glucose limited chemostat culture with 79% CO2 D=0.1/h -2
100	Aerobic glucose limited chemostat culture with 79% CO2 D=0.1/h -3
101	Aerobic nitrogen limited chemostat culture with 79% CO2 D=0.1/h -1
102	Aerobic nitrogen limited chemostat culture with 79% CO2 D=0.1/h -2
103	Aerobic nitrogen limited chemostat culture with 79% CO2 D=0.1/h -3
104	Carbon-limited anaerobic chemostat with benzoate -1
105	Carbon-limited anaerobic chemostat with benzoate -2
106	Carbon-limited anaerobic chemostat with benzoate -3
107	C-lim Anaerobic chemostat dilution rate 0.05h-1 - 1
108	C-lim Anaerobic chemostat dilution rate 0.05h-1 - 2
109	C-lim Anaerobic chemostat dilution rate 0.05h-1 - 3
110	Aerobic galactose C-lim chemostat culture-1
111	Aerobic galactose C-lim chemostat culture-2
112	Aerobic galactose C-lim chemostat culture-3
113	Aerobic galactose C-lim chemostat culture-4
114	Aerobic galactose C-lim chemostat culture-5
115	Aerobic galactose C-lim chemostat culture-6
116	Aerobic S-lim chemostat culture-1
117	Aerobic S-lim chemostat culture-2
118	C-lim Anaerobic chemostat dilution rate 0.03h-1 - 1
119	N-lim Anaerobic chemostat dilution rate 0.03h-1 - 2
120	C-lim Anaerobic chemostat dilution rate 0.2h-1 - 1
121	C-lim Anaerobic chemostat dilution rate 0.2h-1 - 2
122	Aerobic pH3.5 C-lim chemostat culture-1
123	Aerobic pH3.5 C-lim chemostat culture-2
124	Aerobic pH3.5 C-lim chemostat culture-3
125	Aerobic pH6.5 C-lim chemostat culture-1
126	Aerobic pH6.5 C-lim chemostat culture-2
127	Aerobic pH6.5 C-lim chemostat culture-3
128	N-lim Anaerobic chemostat dilution rate 0.2h-1 - 1
129	N-lim Anaerobic chemostat dilution rate 0.2h-1 - 2
130	C-lim Anaerobic chemostat dilution rate 0.03h-1 - 2
131	C-lim Anaerobic chemostat dilution rate 0.03h-1 - 3

132	Acetate limited chemostat culture D=0.1/h -4
133	Ethanol limited chemostat culture D=0.1/h -4
134	N-lim Anaerobic chemostat dilution rate 0.2h-1 - 3
135	N-lim Anaerobic chemostat dilution rate 0.03h-1 - 3
136	N-lim Anaerobic chemostat dilution rate 0.2h-1 - 4
137	Anaerobic carbon limited with methionine as N-source
138	Anaerobic carbon limited with methionine as N-source -2
139	Anaerobic carbon limited with methionine as N-source -3
140	Aerobic glucose limited chemostat with anaerobic factors (tween and ergosterol) -1
141	Aerobic glucose limited chemostat with anaerobic factors (tween and ergosterol) -2
142	Aerobic glucose limited chemostat with anaerobic factors (tween and ergosterol) -3
143	Aerobic phosphorus limited chemostat with anaerobic factors (tween and ergosterol) -1
144	Aerobic phosphorus limited chemostat with anaerobic factors (tween and ergosterol) -2
145	Aerobic phosphorus limited chemostat with anaerobic factors (tween and ergosterol) -3
146	Anaerobic glucose limited chemostat with methionine as sulfur source-1
147	Anaerobic glucose limited chemostat with methionine as sulfur source-2
148	Anaerobic glucose limited chemostat with methionine as sulfur source-3
149	Anaerobic nitrogen limited chemostat with methionine as nitrogen source-1
150	Anaerobic nitrogen limited chemostat with methionine as nitrogen source-2
151	Anaerobic nitrogen limited chemostat with methionine as nitrogen source-3
152	Anaerobic nitrogen limited chemostat with methionine as nitrogen and sulfur source-1
153	Anaerobic nitrogen limited chemostat with methionine as nitrogen and sulfur source-2
154	Anaerobic nitrogen limited chemostat with methionine as nitrogen and sulfur source-3
155	Anaerobic sulfur limited chemostat -1
156	Aerobic glucose limited chemostat with Phenylalanine as N-source-1
157	Aerobic glucose limited chemostat with Phenylalanine as N-source-2
158	Aerobic glucose limited chemostat with leucine as nitrogen source-1
159	Aerobic glucose limited chemostat with leucine as nitrogen source-2
160	Iron limited chemostat cultures with ethanol as a carbon source
161	Iron limited chemostat cultures with ethanol as a carbon source-2
162	Anaerobic nitrogen limited chemostat 0.03h-1 -1
163	Anaerobic glucose limited chemostat
164	Aerobic glucose limited chemostat with 25 mM formate-1
165	Aerobic sulfur limited culture (cDNA from total RNA)-2
166	C-lim aerobic chemostat with 25mM formate
167	CENPK113-7D glucose/ethanol (19mM) limited chemostat -1
168	CENPK113-7D glucose/ethanol (19mM) limited chemostat -2
169	CENPK113-7D glucose/ethanol (19mM) limited chemostat -3
170	C-lim Aerobic chemostat with maltose as C-source 1

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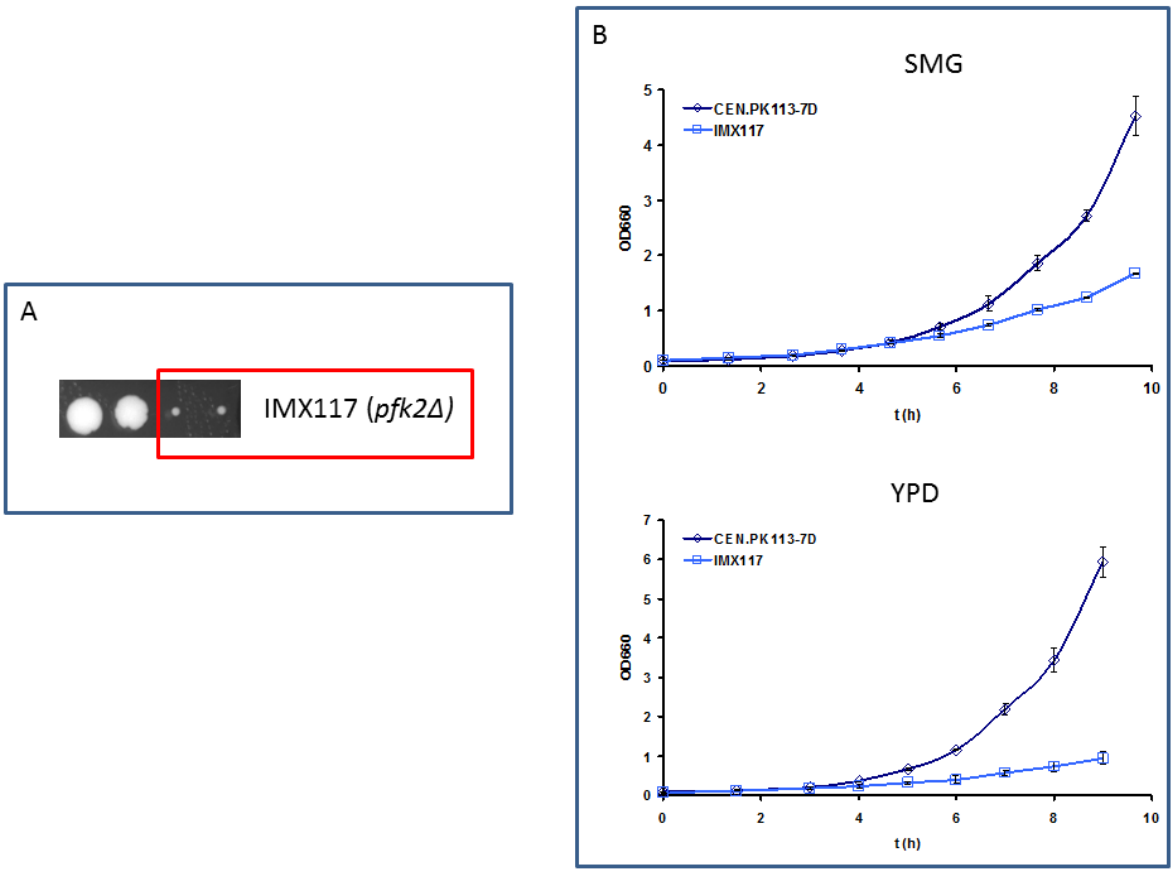

Figure S1. Growth reduction of *pfk2* mutants. *Saccharomyces cerevisiae*'s *pfk2* mutants were obtained by deletion of the *PFK2* gene in the diploid strain CEN.PK122 using a cassette obtained by PCR using Phusion polymerase (Thermo fisher) and the primers 5'-AGAACTAGATTTAGAGACTAGTTTAGCATTGGCCAAGAACTAACCATACGCAATGGATGTCCACGAG CTCTCTACATTGCATGGAATCAGGGCCAGCTGAAGCTTCGTACGC-3' and 5'-TTAACATTAATTGACATTAATAATAGAAAGTGTAATAAAAGGTCATTTTCTTTTACGGTGTCGGTCTC GTAGACGGCGAGTATATGTATGCTGCATAGGCCACTAGTGGATCTG-3' and the plasmid pUG6 as template. CEN.PK112 cells were transformed with the obtained cassette using the lithium-acetate protocol (9). Mutants were then grown in sporulation media and spores were dissected **(A)**. The resulted *pfk2* mutant was termed IMX117 and used to inoculate shake flask contained synthetic media or yeast extract and peptone liquid media with 2% glucose as sole carbon source (SMG and YPD respectively). The changes in optical density at 660 nm

(OD660) of the culture was followed for 9 h **(B)**. The strain CEN.PK113-7D was used as reference strain. The growth of *pfk2* mutants was significantly reduced (ca. 70% of the growth of the reference strain). Data showed is the average of two independent culture replicates, error bars represent the standard deviation.

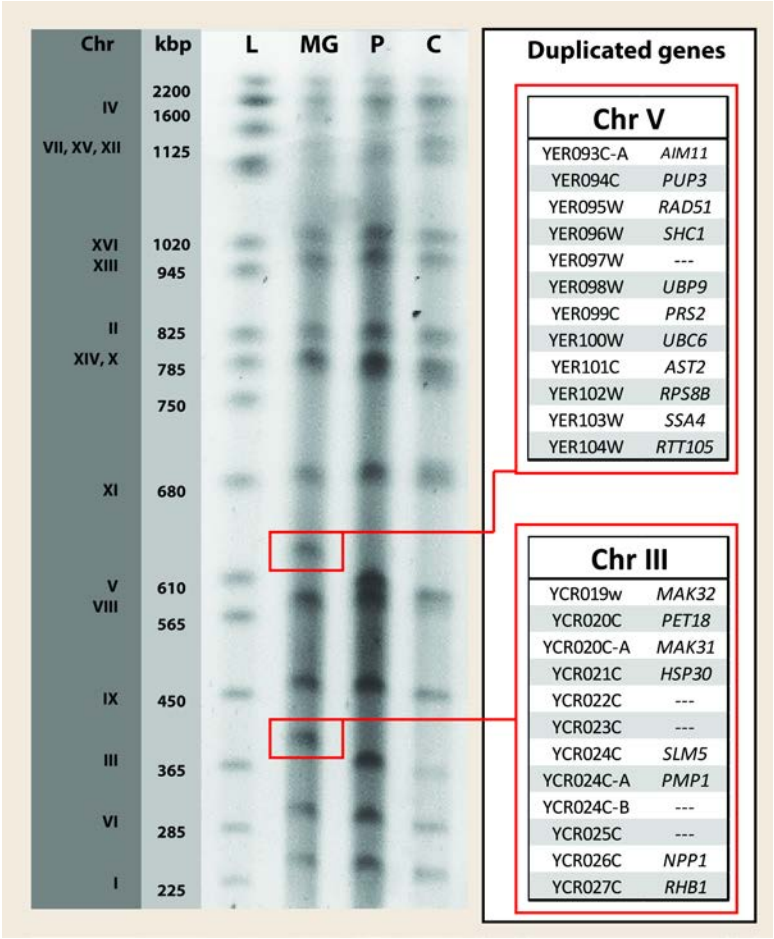

Figure S2. Karyotype of the minimal glycolysis strain (MG), its parental strain CEN.PK102-12A (P) and the reference strain CEN.PK113-7D (C). Chromosomes of the different strains (MG, P and C) were separated using CHEF electrophoresis. Size and chromosome order were identified using the ladder (L) of *S. cerevisiae* YNN295. The size increase of the chromosomes V and III in MG perfectly correlate with the duplication of 12 genes in chromosome V and 12 genes in chromosome III. Agarose Plugs for the different strains were prepared using the

CHEF yeast genomic DNA plugs Kit (Bio-Rad, Richmond, CA), following manufacturer's recommendations, and used for CHEF electrophoresis. CHEF electrophoresis was performed as previously described (7).

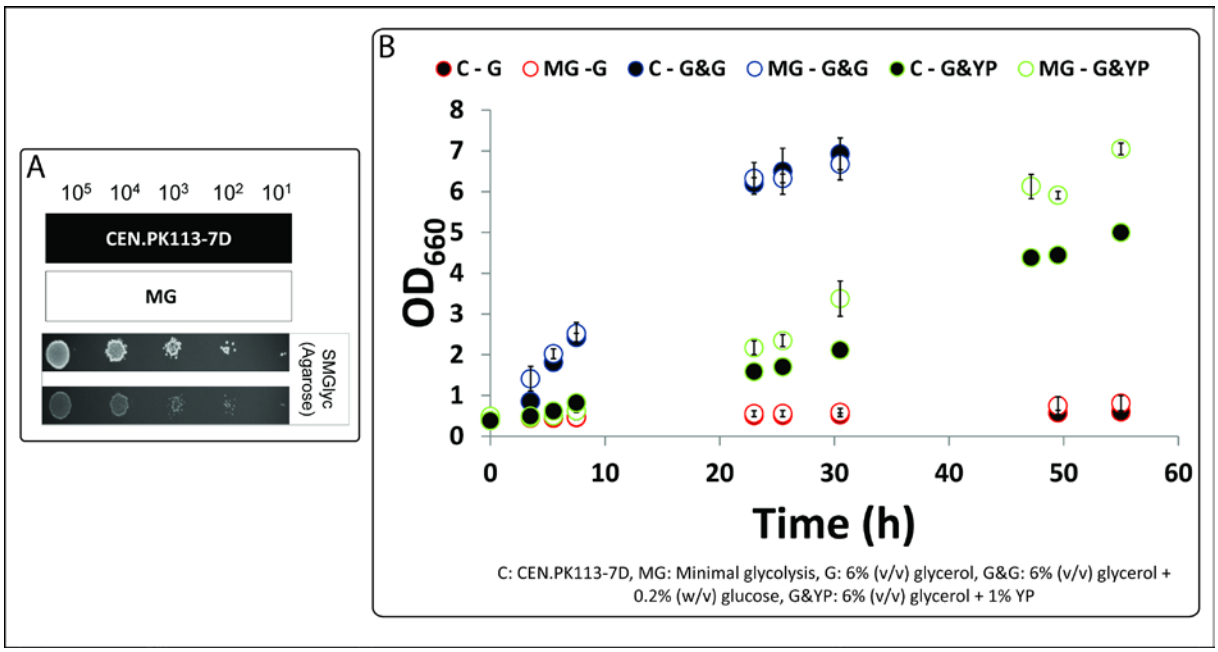

Figure S3. Minimal glycolysis strain (MG) and CEN.PK113-7D (C) growth on media containing glycerol as sole carbon source. A) Serial dilutions of MG (bottom) and CEN.PK113-7D (top) suspensions were inoculated in agarose plates containing synthetic media with 6% (v/v) glycerol as sole carbon source (SMGlyc). **B)** MG (open circles) and CEN.PK113-7D (C, closed circles) were inoculated in shake flasks containing synthetic media with 6% (v/v) glycerol (G, red) and G enriched with 0.2% (v/v) glucose (G&G, blue) or 1% yeast extract and peptone (G&YP, green). Optical density (OD,) measured at 660 nm, of the different cultures was followed as an indication of biomass formation. The data in the plot show the average values of two independent culture replicates for each cultivation condition, the error bar represent the standard deviation.

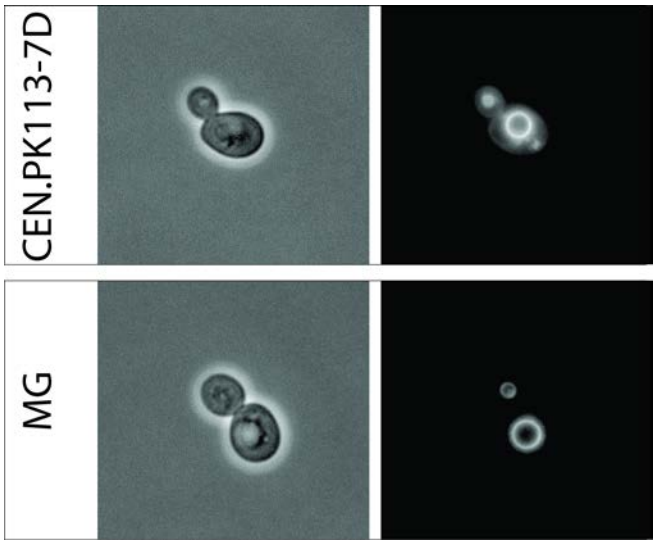

Figure S4. Minimal glycolysis strain (MG) and CEN.PK113-7D vacuoles. MG and CEN.PK113-7D vacuoles were stained with the red fluorescent dye FM4-64 (Excitation/emission ~515/640 nm) (Thermo Fisher Scientific) following manufacturer's recommendations. Yeast cells and vacuoles were visualized with an Imager-Z1 microscope equipped with an AxioCam MR camera, an EC Plan-Neofluar 100x/1.3 Oil Ph 3 M27 objective and the filter set: BP 535/25, FT 580, LP 590 (Carl-Zeiss, Oberkochen, Germany).

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