Supplemental Materials

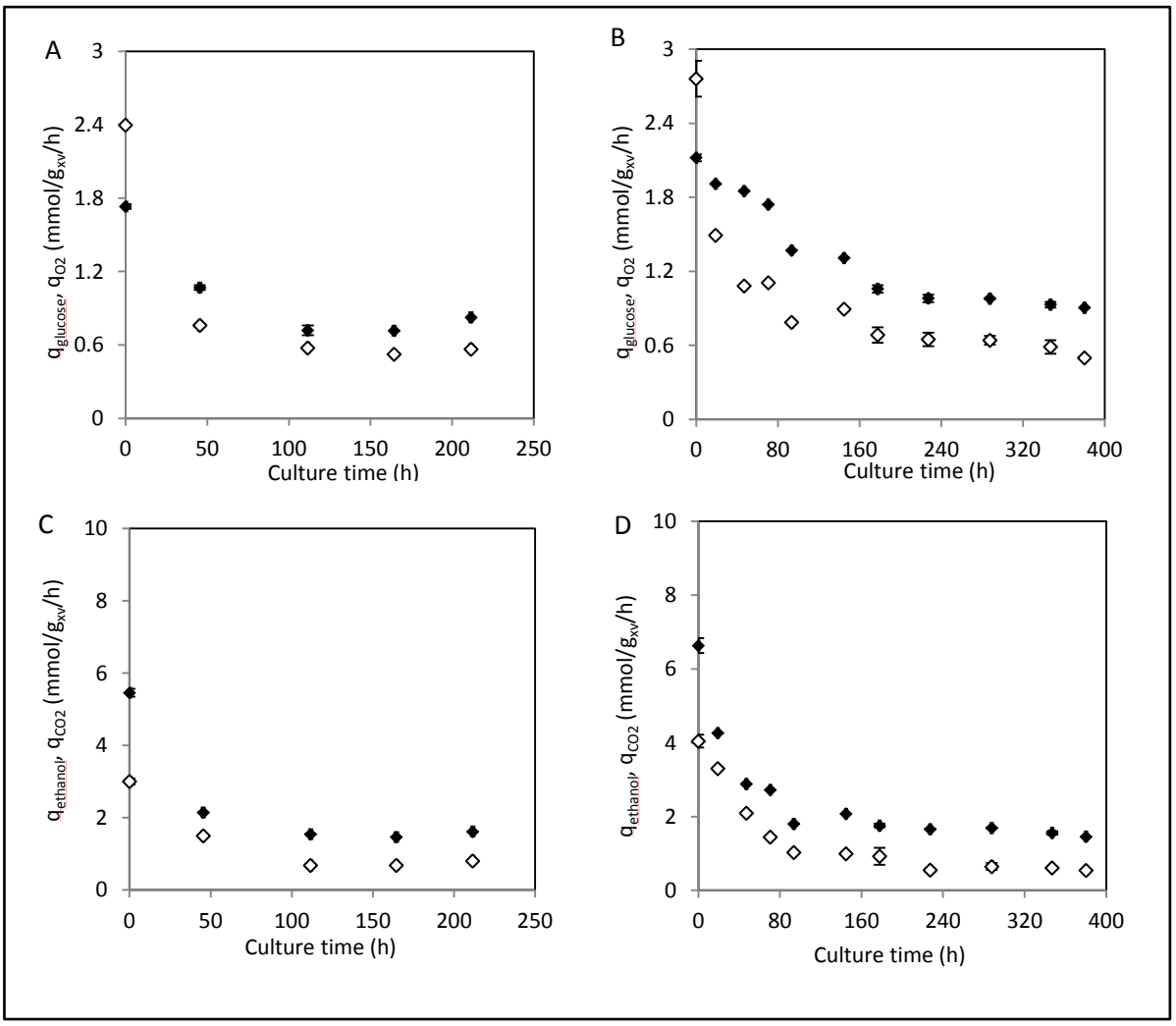

Fig. S1 Biomass-specific consumption rates of glucose and oxygen and production rates of ethanol and carbon dioxide in aerobic ammonium- and phosphate-limited retentostat cultures of *S. cerevisiae* CEN.PK113-7D. Data represent the averages and standard errors of measurements from duplicate retentostat cultures.

A, B: biomass-specific consumption rates of oxygen (closed symbols) and glucose(open symbols) in ammonium-limited (A) and phosphate-limited (B) retentostat cultures.

C, D: biomass-specific production rates of carbon dioxide (closed symbols) and ethanol(open symbols) in ammonium-limited (C) and phosphate-limited (D) retentostat cultures.

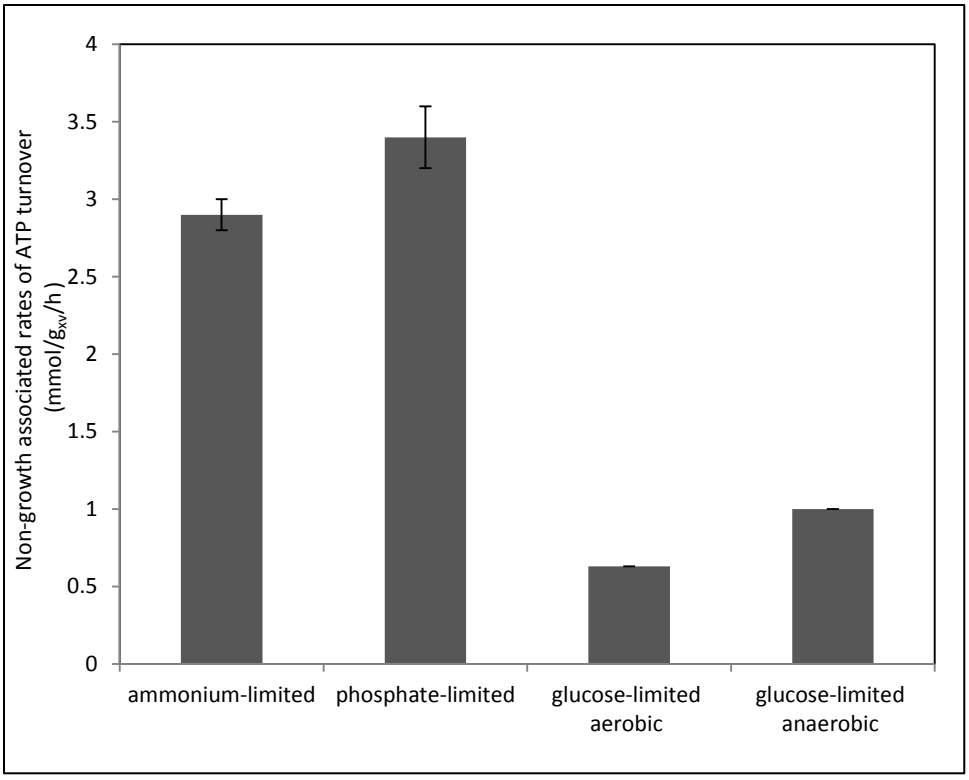

Fig. S2 Non-growth associated rates of ATP turnover in retentostat cultures. Data from glucose-limited aerobic and anaerobic cultures were obtained from previously published work (1, 2). Data from this study represent the averages and standard errors of measurements from duplicate cultures.

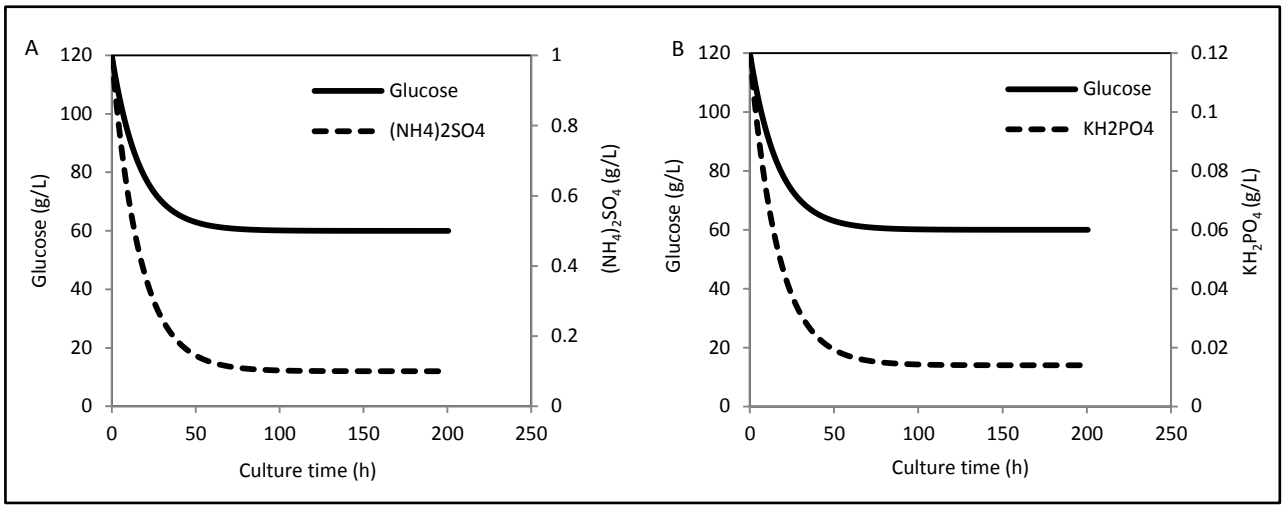

Fig. S3 Gradual switch between chemostat and retentostat media during the start of the retentostat cultivations, (A) feed concentrations of glucose and ammonium sulfate of the N-limited retentostat cultures, (B) feed concentrations of glucose and potassium dihydrogen phosphate of the P-limited retentostat cultures vs culture time in the retentostat.

Table S1 Determination of the required concentrations of $(NH_4)_2SO_4$ and KH_2PO_4 in the feeds of the N- and P-limited retentostats.

total N released from AAs [a] (mol/L)	total N released from protein (mol/L)	total N released rate (mol/g_{xv}/h)	total N compensation rate (based on 5 g L^{-1} biomass) (mol/g_{xv}/h)	$(NH_4)_2SO_4$ (g/L)
6.71E-03	5.71E-04	8.28E-06	4.14E-05	0.10

total P released from metabolites [b] (mol/L)	total P released rate (mol/g_{xv}/h)	total P compensation rate (based on 5 g L^{-1} biomass) (mol/g_{xv}/h)	KH_2PO_4 (g/L)
4.57E-04	5.19E-07	2.59E-06	0.014

a: Measured amino acids: Ala, Gly, Val, Leu, Ile, Pro, Ser, Thr, Met, Asp, Phe, Cys, Glu, Lys, Asn, Gln, Tyr, His, Trp

b: Measured P-containing metabolites: FBP, PEP, G1P, 6PG, T6P, G3P, UDP-Glc, GAP, 2PG, DHAP, 3PG, E4P, Rib5P, Ribu5P, Xyl5P, M6P, F6P, G6P, S7P, AMP,ADP, ATP, CoA, AcCoA

Table S2 Measured viabilities in steady state chemostat and pronged retentostat cultures, data present averages with their standard errors of results from duplicate experiments.

culture condition	culture time (h)	viability from CFU (%)	viability from CFDA stainning (%)	viability from PI stainning (%)
N-limited chemostat	136	80 ± 7	93 ± 2	95 ± 1
	184	87 ± 6	90 ± 0	93 ± 0
	256	84 ± 2	92 ± 2	92 ± 0
N-limited retentostat	0	82 ± 3	92 ± 2	92 ± 0
	43	81 ± 1	91 ± 2	90 ± 0
	70	79 ± 0	85 ± 0	88 ± 0
	117	71 ± 5	83 ± 1	87 ± 0
	170	66 ± 4	81 ± 1	84 ± 1
	213	67 ± 5	79 ± 1	78 ± 2
P-limited chemostat	103	80 ± 27	92 ± 3	93 ± 1
	153	80 ± 7	92 ±4	92 ± 0
	201	75 ± 2	87 ± 7	90 ± 2
P-limited retentostat	0	72 ± 12	89 ± 10	90 ± 0
	33	70 ± 30	89 ± 8	88 ± 2
	79	59 ± 11	84 ± 3	86 ± 1
	152	61 ± 11	83 ± 5	84 ± 2
	201	63 ± 25	80 ± 7	83 ± 1
	249	61 ± 0	82 ± 4	82 ± 1
	321	61 ± 10	77 ± 5	81 ± 1
	354	62 ± 5	76 ± 9	85 ± 0
	382	62 ± 8	76 ± 12	80 ± 0

Table S3 Input parameters for metabolic flux analysis (S3A) and obtained flux values with their standard errors of central metabolism (S3B) for aerobic ammonium- and phosphate-limited (N- and P- limited) steady-state, slow growth (SG) ($\mu = 0.025\ h^{-1}$) chemostat cultures, and pseudo-steady-state, near-zero growth (NZG) ($\mu < 0.002\ h^{-1}$) retentostat cultures of *S. cerevisiae* CEN.PK113-7D.

Table S3A

Input parameters	Unit	N-limited at SG	N-limited at NZG	P-limited at SG	P-limited at NZG
q_X	$mmol/g_{xv}/h$	0.96 ± 0.09	0.022 ± 0.003	0.99 ± 0.04	0.017 ± 0.003
q_{CO2}	$mmol/g_{xv}/h$	5.5 ± 0.2	1.5 ± 0.0	6.6 ± 0.3	1.6 ± 0.1
$q_{ethanol}$	$mmol/g_{xv}/h$	3.1 ± 0.1	0.75 ± 0.00	4.0 ± 0.2	0.55 ± 0.05
$q_{glucose}$	$mmol/g_{xv}/h$	2.3 ± 0.0	0.57 ± 0.01	2.7 ± 0.2	0.53 ± 0.01
q_{O2}	$mmol/g_{xv}/h$	1.7 ± 0.0	0.71 ± 0.03	2.1 ± 0.0	0.94 ± 0.02
μ	h^{-1}	0.025 ± 0.002	0.00054 ± 0.00006	0.028 ± 0.003	0.00042 ± 0.0008
biomass protein	% (g/[100 g biomass])	12.6 ± 0.2	9.6 ± 0.4	32.3 ± 1.7	26.3 ± 2.1
Mw	g/mol	25.6 ± 0.0	24.3 ± 0.3	27.3 ± 0.2	25.4 ± 0.1
C molar formulas		$CH_{1.8}O_{0.63}N_{0.063}P_{0.011}S_{0.0013}$	$CH_{1.8}O_{0.60}N_{0.042}P_{0.0082}S_{0.0009}$	$CH_{1.9}O_{0.63}N_{0.10}P_{0.0044}S_{0.0034}$	$CH_{1.8}O_{0.59}N_{0.083}P_{0.0023}S_{0.0022}$

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Metabolic Reaction	Enzyme	N-limited at SG mmol/g_{xv}/h	P-limited at SG mmol/g_{xv}/h	N-limited at NZG mmol/g_{xv}/h	P-limited at NZG mmol/g_{xv}/h
	Glycolysis				
Glucose→G6P	hexokinase	2.28 ± 0.04	2.67 ± 0.04	0.50 ± 0.01	0.48 ± 0.00
G6P→F6P	G6P isomerase	2.12 ± 0.03	2.51 ± 0.04	0.50 ± 0.01	0.48 ± 0.00
F6P→F16BP	phosphofructokinase	2.15 ± 0.03	2.54 ± 0.04	0.50 ± 0.01	0.48 ± 0.00
F16BP→DHAP+GAP	fructosebisphosphate aldolase	2.15 ± 0.03	2.54 ± 0.04	0.50 ± 0.01	0.48 ± 0.00
DHAP→GAP	triosephosphate isomerase	2.15 ± 0.03	2.54 ± 0.04	0.50 ± 0.01	0.48 ± 0.00
GAP→13BPG	glyceraldehyde phosphate dehydrogenase	4.31 ± 0.06	5.09 ± 0.08	0.99 ± 0.01	0.96 ± 0.01
13BPG→3PG	phosphoglycerate kinase	4.31 ± 0.06	5.09 ± 0.08	0.99 ± 0.01	0.96 ± 0.01
3PG→2PG	phosphoglycerate mutase	4.30 ± 0.06	5.08 ± 0.08	0.99 ± 0.01	0.96 ± 0.01
2PG→PEP	enolase	4.30 ± 0.06	5.08 ± 0.08	0.99 ± 0.01	0.96 ± 0.01
PEP→PYR	pyruvate kinase	4.30 ± 0.06	5.07 ± 0.08	0.99 ± 0.01	0.96 ± 0.01
	PP-pathway				
G6P→6PG	G6P dehydrogenase	0.05 ± 0.00	0.05 ± 0.00	0.0012 ± 0.0001	0.0009 ± 0.0002
6PG→RIBU5P	6-phosphogluconate dehydrogenase	0.05 ± 0.00	0.05 ± 0.00	0.0012 ± 0.0001	0.0009 ± 0.0002
RIBU5P→RIB5P	ribosephosphate isomerase	0.02 ± 0.00	0.02 ± 0.00	0.0004 ± 0.0000	0.0003 ± 0.0001
RIBU5P→XYL5P	ribulosephosphate epimerase	0.03 ± 0.00	0.03 ± 0.00	0.0007 ± 0.0001	0.0005 ± 0.0001
RIB5P+XYL5P→GAP+SED7P	transketolase 1	0.02 ± 0.00	0.02 ± 0.00	0.0004 ± 0.0000	0.0003 ± 0.0001
GAP+SED7P→E4P+S6P	transaldolase	0.02 ± 0.00	0.02 ± 0.00	0.0004 ± 0.0000	0.0003 ± 0.0001
RIB5P+XYL5P→GAP+SED7P	transketolase 2	0.01 ± 0.00	0.02 ± 0.00	0.0003 ± 0.0000	0.0002 ± 0.0000
	TCA-cycle				
ACCOA+OAA→COA+CIT	citrate synthase mitochondrial	0.59 ± 0.01	0.66 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
CIT→ISOCIT	aconitase 1,2	0.59 ± 0.01	0.66 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
ICIT→AKG	isocitrate dehydrogenase (NAD)	0.58 ± 0.01	0.65 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
ICIT→AKG	isocitrate dehydrogenase (NADP)	0.01 ± 0.00	0.01 ± 0.00	0.0002 ± 0.0000	0.0001 ± 0.0000
AKG→SUCCOA	a-ketoglutarate dehydrogenase	0.58 ± 0.01	0.65 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
SUCCOA→SUC	succinylcoa synthetase	0.58 ± 0.01	0.65 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
SUC→FUM	succinate dehydrogenase	0.58 ± 0.01	0.65 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
FUM→MAL	fumarate hydrase	0.58 ± 0.01	0.65 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
FUM→MAL	fumarate hydrase cytosolic	0.01 ± 0.00	0.01 ± 0.00	0.0001 ± 0.0000	0.0001 ± 0.0000
MAL→OAA	malate dehydrogenase	0.59 ± 0.01	0.66 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
	Pyruvate branchpoint				
PYR→ACT	pyruvate decarboxylase	3.67 ± 0.07	4.21 ± 0.20	0.73 ± 0.00	0.61 ± 0.01
PYR→ACCOA	pyruvate dehydrogenase	0.59 ± 0.01	0.66 ± 0.06	0.27 ± 0.01	0.35 ± 0.02
PYR→OAA	pyruvate carboxylase	0.02 ± 0.00	0.02 ± 0.00	0.0004 ± 0.0000	0.0003 ± 0.0001
ACT→ETOH	alcohol dehydrogenase	3.61 ± 0.06	4.14 ± 0.20	0.72 ± 0.00	0.61 ± 0.01
ACT→ACCOA	acetyl-CoA synthase	0.06 ± 0.00	0.06 ± 0.00	0.001 ± 0.000	0.001 ± 0.000
ACCOA→COA	acetylcoa carboxylase cyt	0.05 ± 0.00	0.05 ± 0.00	0.001 ± 0.000	0.001 ± 0.000
ATP→ADP	**Non-growthed associated rates of ATP turnover**	8.4 ± 0.4	10.9 ± 0.5	2.9 ± 0.1	3.4 ± 0.2

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Table S4 Extracellular amino acids concentration in aerobic ammonium-limited (N-limited) steady-state, slow growth (SG) ($\mu = 0.025\ h^{-1}$) chemostat cultures, and pseudo-steady-state, near-zero growth (NZG) ($\mu < 0.002\ h^{-1}$) retentostat cultures of *S. cerevisiae* CEN.PK113-7D. Data represent the averages and standard errors of multiple measurements from duplicate cultures.

	N-limited at SG	N-limited at NZG	Km from literature
	μM	μM	μM
Ala	17.9 ± 4.4	6.9 ± 1.3	37(3)
Gly	0.90 ± 0.25	0.46 ± 0.02	~[a]
Val	2.0 ± 0.3	0.81 ± 0.06	37(3)
Leu	0.47 ± 0.05	1.2 ± 0.3	37(3, 4)
Ile	0.31 ± 0.05	0.59 ± 0.19	37(3)
Pro	0.17 ± 0.03	0.10 ± 0.04	34(5, 6)
Ser	1.1 ± 0.1	1.2 ± 0.2	590(3)
Thr	0.72 ± 0.09	0.57 ± 0.02	590(3)
Meth	~[b]	~[b]	13(7)
Asp	1.0 ± 0.1	1.0 ± 0.0	56(3)
Phe	0.38 ± 0.03	0.92 ± 0.31	24(8)
Cys	25.9 ± 3.9	4.4 ± 0.6	37, 55(3, 9)
Glu	8.8 ± 0.8	4.2 ± 0.5	48(3)
Lys	33.7 ± 2.2	0.52 ± 0.17	10-25(3, 10)
Asn	~[c]	3.5 ± 0.0	590(3)
Gln	4.1 ± 0.6	6.0 ± 0.3	590(3, 11)
Tyr	~[d]	~[d]	160(12)
His	~[e]	0.11 ± 0.06	10-20(3, 13)
Trp	~[f]	~[f]	42(14)

a: did not find in literature

b: Meth was below the detection limit of assay: 0.03 μM

c: Asn was below the detection limit of assay: 0.125 μM

d: Tyr was below the detection limit of assay: 0.03 μM

e: His was below the detection limit of assay: 0.12 µM

f: Trp was below the detection limit of assay: 0.025 µM

Table S5 Comparison of respiratory quotients (RQ) of aerobic ammonium- and phosphate-limited steady state, slow growth (SG) ($\mu = 0.025\ h^{-1}$) chemostat cultures and pseudo-steady-state, near-zero growth (NZG) ($\mu < 0.002\ h^{-1}$) retentostat cultures of *S. cerevisiae* CEN.PK113-7D. Data from this study represent averages with standard errors of measurements from duplicate cultures.

μ (h^{-1})	RQ		Reference
	ammonium-limited	phosphate-limited	
0.1	4.5 ± 0.2	3.4 ± 0.0	Boer's study (15)
0.025	3.1 ± 0.0	3.1 ± 0.1	This study
< 0.002	2.1 ± 0.1	1.7 ± 0.0	This study

Table S6 Nitrogen balance of N-limited retentostats at near-zero specific growth rates

N balance based on "Total N"		N balance based on "AAs and protein"	
qn_{in} (mgN/gX/h)	0.057 ± 0.009	qn_{in} (mgN/gX/h)	0.057 ± 0.009
qn_{out} (mgN/gX/h)	0.033 ± 0.000	qn_{AAs} (mgN/gX/h)	0.008 ± 0.004
		$qn_{protein}$ (mgN/gX/h)	0.031 ± 0.006
qn_X (mgN/gX/h)	0.022 ± 0.007	qn_X (mgN/gX/h)	0.022 ± 0.007
N recovery (%)	98 ± 3	N recovery (%)	102 ± 1

Reference

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