

Additional file (Vanz et al 2014)

Table S1 - All identified intracellular proteins of *P. pastoris* X-33 classified into functional categories

NCBI Accession-No ¹	Locus ID ¹	Gene name ²	Protein name ²	MW (kDa) ³	pI ⁶	Mascot Score	Spot No ⁴
Functional category¹							
1. Metabolism							
1.1 Carbohydrate metabolism							
Methanol metabolism							
XP_002494271	PAS_chr4_0821	<i>AOX1</i>	Alcohol oxidase	74.5	6.0	124	14*, 15*, 148*, 156* (74.5 kDa), 163* (~25 kDa)
XP_002492075	PAS_chr2-2_0131	<i>CTA1</i>	Catalase A	58.1	6.6	183	18*
XP_002493065	PAS_chr3_0832	<i>DAS1</i>	Transketolase (Dihydroxyacetone synthase), similar to Tkl2p	79.1	6.0	174	153*, 223*, 16*
XP_002493071	PAS_chr3_0841	<i>DAK</i>	Dihydroxyacetone kinase	65.4	5.4	114	57*, 58*
XP_002493270	PAS_chr3_1028	<i>FLD1</i>	S-(hydroxymethyl) glutathione dehydrogenase	41.4	6.1	81 ⁵	24*, 25*, 28*
XP_002493100	PAS_chr3_0867	<i>FGH1</i>	Non-essential intracellular esterase that can function as an S-formylglutathione hydrolase	33.4	6.3	131	49*, 54*
XP_002493171	PAS_chr3_0932	<i>FDH1</i>	NAD(+)-dependent formate dehydrogenase	40.4	6.6	119	29*, 30*, 31*, 32*, 33* (40 kDa), 61* (~10 kDa), 80*, 81* (~17 kDa), 87*, 89* (~30 kDa)
Glycolysis							
XP_002494228	PAS_chr4_0783	<i>GUT1</i>	Glycerol kinase	68.8	5.3	104	145*

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Functional category¹							
XP_002493103	PAS_chr3_0868	<i>FBP1</i>	Fructose-1,6-bisphosphatase	38.4	6.1	118	41*
XP_002491345	PAS_chr2-1_0437	<i>TDH1</i>	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3	35.7	6.2	106	45*, 48*, 95*, 118*
XP_002489713	PAS_chr1-1_0072	<i>FBA1</i>	Fructose 1,6-bisphosphate aldolase	39.9	6.0	186	115*
XP_002490411	PAS_chr1-4_0292	<i>PGK1</i>	3-Phosphoglycerate kinase	44.1	7.8	152	210*, 211*
XP_002493191	PAS_chr3_0951	<i>TPI1</i>	Triose phosphate isomerase	27.1	5.7	244	112*
XP_002493059	PAS_chr3_0826	<i>GMP1</i>	Tetrameric phosphoglycerate mutase	28.0	6.0	165	65*
XP_002492293	PAS_chr3_0082	<i>ENO2</i>	Enolase I	46.5	5.4	102	22*, 59*, 150*
Ethanol metabolism							
CAY67035	PAS_c034_0018	<i>ADH</i>	Alcohol dehydrogenase	38.1	6.3	195	78*
XP_002491382	PAS_chr2-1_0472	<i>ADH3</i>	Mitochondrial alcohol dehydrogenase isozyme III	37.3	5.8	145	42*, 44*, 55*, 93*, 96*
Citrate cycle (TCA cycle)							
XP_002489764	PAS_chr1-1_0475	<i>CIT1</i>	Citrate synthase	51.9	7.8	102	27*
XP_002489444	PAS_chr1-3_0104	<i>ACO1</i>	Aconitase	85.0	5.7	77	129*
XP_002491128	PAS_chr2-1_0238	<i>MDH1</i>	Mitochondrial malate dehydrogenase	36.5	5.3	129	43*, 47*, 53*, 56*, 103*, 141*
Pentose phosphate pathway							
XP_002492495	PAS_chr3_0277	<i>6PGD [GND]</i>	6-Phosphogluconate dehydrogenase (decarboxylating)	54.2	5.9	116	23*
XP_002491849	PAS_chr2-2_0337	<i>TAL1</i>	Transaldolase	35.7	5.1	111	109*
Sugar metabolism and others							
XP_002490557	PAS_chr1-4_0426	<i>BGL2</i>	Endo-beta-1,3-glucanase	34.2	4.1	109	158*
XP_002493105	PAS_chr3_0870	<i>VIG9 [PSA1]</i>	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanyltransferase)	40.1	6.0	95	215*

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Functional category ¹							
Pyruvate metabolism							
XP_002492397	PAS_chr3_0188	<i>PDC1</i>	Major of three pyruvate decarboxylase isozymes	61.5	5.6	118	11*, 12*
XP_002491703	PAS_chr2-1_0769	<i>PYK2</i>	Pyruvate kinase	55.9	6.1	190	161*
1.2 Energy Metabolism							
Oxidative phosphorylation							
XP_002493409	PAS_c131_0021	<i>[VMA2]</i>	Vacuolar ATP synthase subunit B	55.4	5.3	159	167*
XP_002492803	PAS_chr3_0576	<i>atpA</i>	Alpha subunit of the F1 sector of mitochondrial F1F0 ATP synthase	59.0	9.1	76	52*, 66*, 125*
XP_002492039	PAS_chr2-2_0165	<i>atpD</i>	Beta subunit of the F1 sector of mitochondrial F1F0 ATP synthase	54.0	5.2	83	6*, 35*, 68*
XP_002494179	PAS_chr4_0737	<i>MCR1</i>	Mitochondrial NADH-cytochrome b5 reductase	33.6	8.8	151	67*
1.3 Nucleotide metabolism							
XP_002492154	PAS_chr2-2_0059	<i>YNK1</i>	Nucleoside diphosphate kinase	17.0	6.2	83	121*
1.4 Amino acid metabolism							
XP_002490090	PAS_chr1-1_0432	<i>ILV5</i>	Acetohydroxyacid reductoisomerase	44.4	7.7	92	37*
XP_002491046	PAS_chr2-1_0160	<i>metE [MET6]</i>	Cobalamin-independent methionine synthase	85.9	5.9	193	107*, 128*
XP_002492638	PAS_chr3_0410	<i>CAR2</i>	L-Ornithine transaminase	47.5	5.9	249	200*
XP_002490747	PAS_chr1-4_0608	<i>ARO8</i>	Aromatic aminotransferase I	54.2	5.4	172	165*
XP_002492166	PAS_chr2-2_0048		Dihydrolipoamide dehydrogenase	52.6	6.3	113	13*
XP_002493126	PAS_chr3_0890	<i>SAHH</i>	S-adenosyl-L-homocysteine hydrolase	49.2	5.4	147	151*
XP_002493112	PAS_chr3_0876	<i>SAM2</i>	S-adenosylmethionine synthetase	42.6	6.1	96	205*

NCBI Accession-No ¹	Locus ID ¹	Gene name ²	Protein name ²	MW (kDa) ³	pI ³	Mascot Score	Spot No ⁴
Functional category¹							
1.5 Metabolism of cofactors and vitamins							
XP_002493470	PAS_chr4_0065	<i>THI5</i>	Protein involved in synthesis of the thiamine precursor hydroxymethylpyrimidine (HMP)	38.7	6.3	94	46*
XP_002493989	PAS_chr4_0550	-	Pyridoxine 4-dehydrogenase	36.3	5.6	97	138*
XP_002492875	PAS_chr3_0648	<i>THI4</i>	Thiazole synthase	37.3	6.2	92	113*
2. Genetic information processing							
2.1 Transcription and translation							
XP_002491688	PAS_chr2-1_0755	<i>EGD2</i>	Alpha subunit of the heteromeric nascent polypeptide-associated complex (NAC)	21.6	4.6	78 ⁵	73*
XP_002489404	PAS_chr1-3_0068	<i>RPP0</i>	Conserved ribosomal protein P0 similar to rat P0, human P0, and E. coli L10e	33.7	4.6	128	72*
XP_002491277	PAS_chr2-1_0376	<i>EFB1</i>	Putative GTPase, member of the Obg family	23.2	4.3	122	108*
XP_002490107	PAS_chr1-1_0449	<i>PST2</i>	Protein with similarity to members of a family of flavodoxin-like proteins	20.9	6.4	73	86*
XP_002493447	PAS_chr4_0041	-	Protein component of the large (60S) ribosomal subunit	15.1	5.8	86	94*
XP_002489653	PAS_chr1-1_0019	<i>SUB2</i>	Component of the TREX complex required for nuclear mRNA export	49.6	5.4	91	166*
XP_002490604	PAS_chr1-4_0471	<i>RPS0</i>	Protein component of the small (40S) ribosomal subunit, nearly identical to Rps0Bp	29.3	4.6	72	71*
XP_002493313	PAS_chr3_1071	<i>CAM1</i>	Translation elongation factor EF-1 gamma	24.3	6.4	104	78*, 137*
XP_002493749	PAS_chr4_0327	<i>GCN1</i>	Positive regulator of the Gcn2p kinase activity, forms a complex with Gcn20p	30.4	5.6	79	101*
2.2 Sorting and degradation							
XP_002492588	PAS_chr3_0365	<i>SSC1</i>	Mitochondrial matrix ATPase	69.7	5.4	152	106*
XP_002490239	PAS_chr1-4_0130	<i>HSP90</i>	Heat shock protein Hsp90	80.9	4.8	81	1*, 2*
XP_002489443	PAS_chr1-3_0102	<i>clpB [HSP104]</i>	Heat shock protein that cooperates with Ydj1p (Hsp40) and Ssa1p (Hsp70)	100.6	5.3	140	315*

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Functional category¹							
<u>XP002494360.1</u>	<u>PAS_FragD_0026</u>	<i>[CDC48]</i>	ATPase in ER, nuclear membrane and cytosol with homology to mammalian p97	91.4	4.8	108	146*
<u>CCA38590</u>	<u>PP7435Chr2-0908</u>	<i>AAP1</i>	Aminopeptidase N	23.0	4.7	142	316*
<u>XP_002493087</u>	<u>PAS_chr3_0856</u>	<i>UBA1</i>	Ubiquitin activating enzyme (E1)	115.6	5.0	142	325*
2.3 Folding catalysts							
<u>CAC33587</u>	<u>PAS_chr4_0844</u>	<i>PDI</i>	Protein disulfide isomerase	58.1	4.6	161	134*
<u>XP_002493562</u>	<u>PAS_chr4_0158</u>	<i>CPNA [HSP60]</i>	Tetradecameric mitochondrial chaperonin	60.5	5.1	115	4*
<u>XP_002489608</u>	<u>PAS_chr1-3_0264</u>	<i>[CPR1]</i>	Cytoplasmic peptidyl-prolyl cis-trans isomerase (cyclophilin)	18.1	6.1	99	92*
<u>XP_002493991</u>	<u>PAS_chr4_0552</u>	<i>HSP70-HSA1 [SSA1]</i>	ATPase involved in protein folding and nuclear localization signal (NLS)-directed	69.7	4.1	89	207*
<u>XP_002492959</u>	<u>PAS_chr3_0731</u>	<i>HSP70 [SSB]</i>	Cytoplasmic ATPase that is a ribosome-associated molecular chaperone	66.6	5.1	100	209*
<u>XP_002491027</u>	<u>PAS_chr2-1_0140</u>	<i>KAR2</i>	ATPase involved in protein import into the ER	74.2	4.8	146	133*
<u>XP_002492443</u>	<u>PAS_chr3_0230</u>	<i>HSP70 [SSA3]</i>	ATPase involved in protein folding and the response to stress	70.9	5.1	102	76* (70.9 kDa) 105* (~25 kDa)
3. Cellular processes							
3.1 Transport and catabolism							
<u>XP_002490181</u>	<u>PAS_chr1-4_0071</u>	<i>SOD2</i>	Mitochondrial superoxide dismutase	25.2	7.9	85	216*
<u>XP_002493819</u>	<u>PAS_chr4_0391</u>	<i>[TOM40]</i>	Component of the TOM (translocase of outer membrane) complex	42.8	5.6	78	36*
<u>XP_002492446</u>	<u>PAS_chr3_1169</u>	<i>ACT1</i>	Actin	41.9	5.3	148	21*
3.2 Cell growth and death							
<u>XP_002490987</u>	<u>PAS_chr2-1_0809</u>	<i>BMH1</i>	14-3-3 protein homolog	29.1	4.8	85	75*
<u>XP_002492031</u>	<u>PAS_chr2-2_0172</u>	<i>NAP1</i>	Protein that interacts with mitotic cyclin Clb2p	49.0	4.3	115	135*

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Functional category ¹							
4. Other							
4.1 Stress response							
XP_002491977	PAS_chr2-2_0220	<i>TSA1</i>	Thioredoxin peroxidase	21.5	4.9	125	77*
XP_002490091	PAS_chr1-1_0433	<i>PRX1</i>	Mitochondrial peroxiredoxin (1-Cys Prx) with thioredoxin peroxidase activity	25.1	5.7	142	82*, 111*
BAH80186	PAS_chr2-2_0480	<i>TRR1</i>	Thioredoxin reductase 1	35.3	5.5	93	225*
XP_002491413	PAS_chr2-1_0502	-	Thiol-specific peroxiredoxin	19.3	8.6	106	120*
5. Unclassified							
XP_002491418	PAS_chr2-1_0853	-	Hypothetical protein	57.0	6.0	84	7*, 8*, 91*
XP_002489760	PAS_chr1-1_0118	-	Laminin subunit gamma-1	46.3	7.0	73	142*, 157*
XP_002491208	PAS_chr2-1_0313	-	Bifunctional enzyme with alcohol dehydrogenase and glutathione-dependent formaldehyde dehydrogenase	39.1	5.8	92	38*, 39*, 40*
XP_002490174	PAS_chr1-4_0063	-	G-protein beta subunit and guanine nucleotide dissociation inhibitor for Gpa2p	35.0	6.3	104	116*
XM_002490789	PAS_FragB_0024	-	Inositol 2-dehydrogenase	38.0	5.7	79	139*

1 Accession numbers and locus IDs are according to the NCBI Reference Sequence database (<http://www.ncbi.nlm.nih.gov/RefSeq/>). Functional classifications are mostly according to KEGG PATHWAY database (<http://www.genome.jp/kegg/metabolism.html>). The data are according to databases on 01.10.12.

2 Gene/protein names are according to the sequenced genome of *P. pastoris* GS115 (<http://www.uniprot.org/>). If no name was found for *P. pastoris* GS115, a Blast search of the respective *P. pastoris* GS115 gene/protein against different *P. pastoris* strains was carried out in the following order: search against ATCC 76273 / CBS 7435 / CECT 11047 / NRRL Y-11430 / Wegner 21-1 and *P. pastoris* (yeast). Gene/protein names from other *P. pastoris* strains were only adopted in case of 100% sequence identity (<http://www.uniprot.org/>). Gene names in brackets are from *S. cerevisiae* if the names differ for both yeasts (<http://www.uniprot.org/>). The data are according to database on 01.10.12.

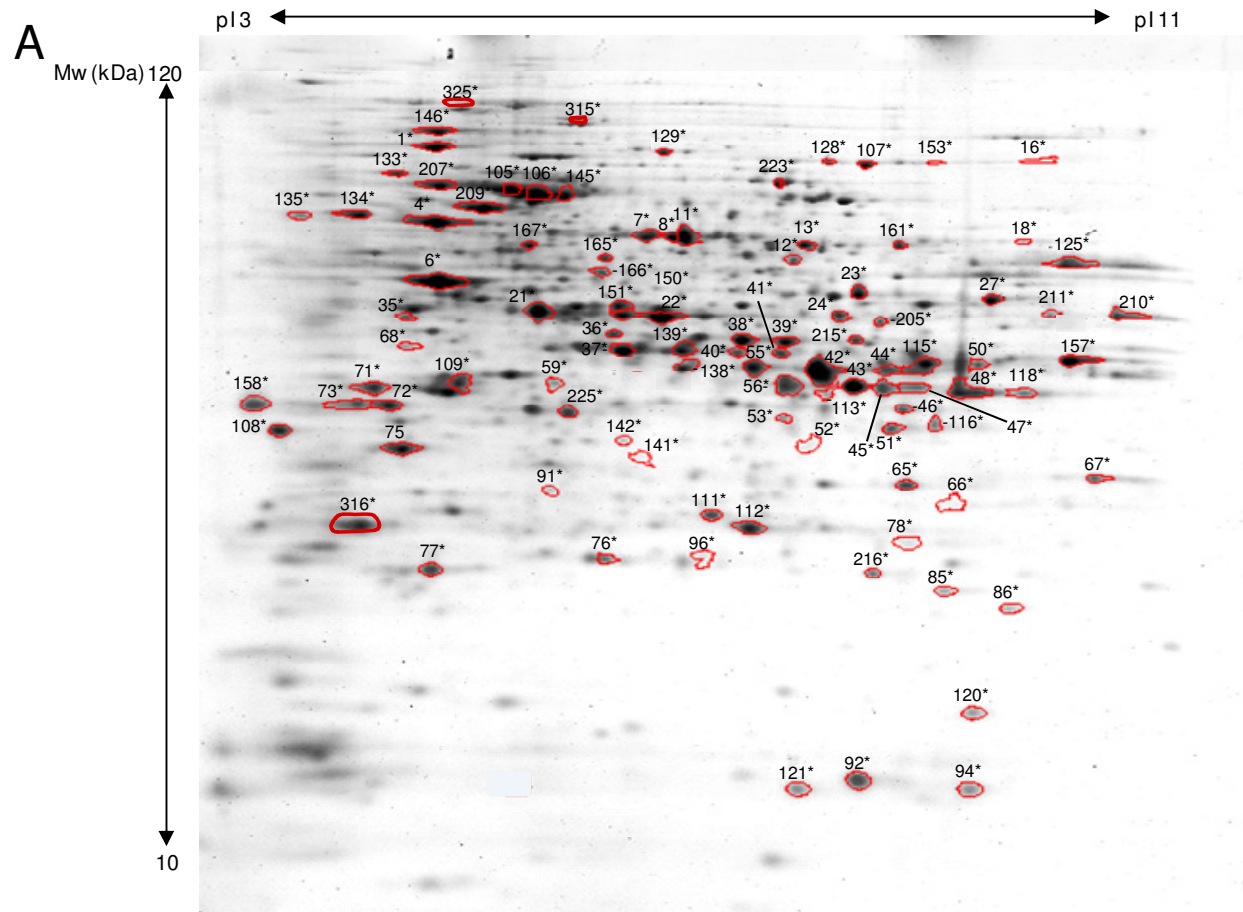
3 Theoretical molecular mass (MM) and isoelectric point (pI) not considering potential posttranslational modifications.

4 "Spot-No" indicates the serial number of all identified protein spots of the intracellular proteome of *P. pastoris* X-33. Proteins appearing in multiple spots indicate the existence of isoforms which probably derive from posttranslational modification events such as phosphorylation, glycosylation or limited proteolysis. Two representative 2D gels of the intracellular proteome from cell samples taken at the end of glycerol phase and after 48 hours of growth on methanol with the respective spot numbers are shown in Figure S1 A and B. Spot numbers in this table and the Figure S1 A and B are marked by an asterisk (*) for simplified searchability. Fragments of full-length proteins, AOX1, FDH1 and SSA3, were identified and the respective Spot No and MW are indicated.

5 Score obtained from MS/MS analysis.

Figure S1 A and B - Representative 2D gels of the intracellular proteome before and during IP production

The samples of *P. pastoris* X-33 were taken at the end of the glycerol phase (A) and after 48 h of growth on methanol (B). Spot numbers correspond to numbers in the Table S1 and are marked by an asterisk (*) for simplified searchability.



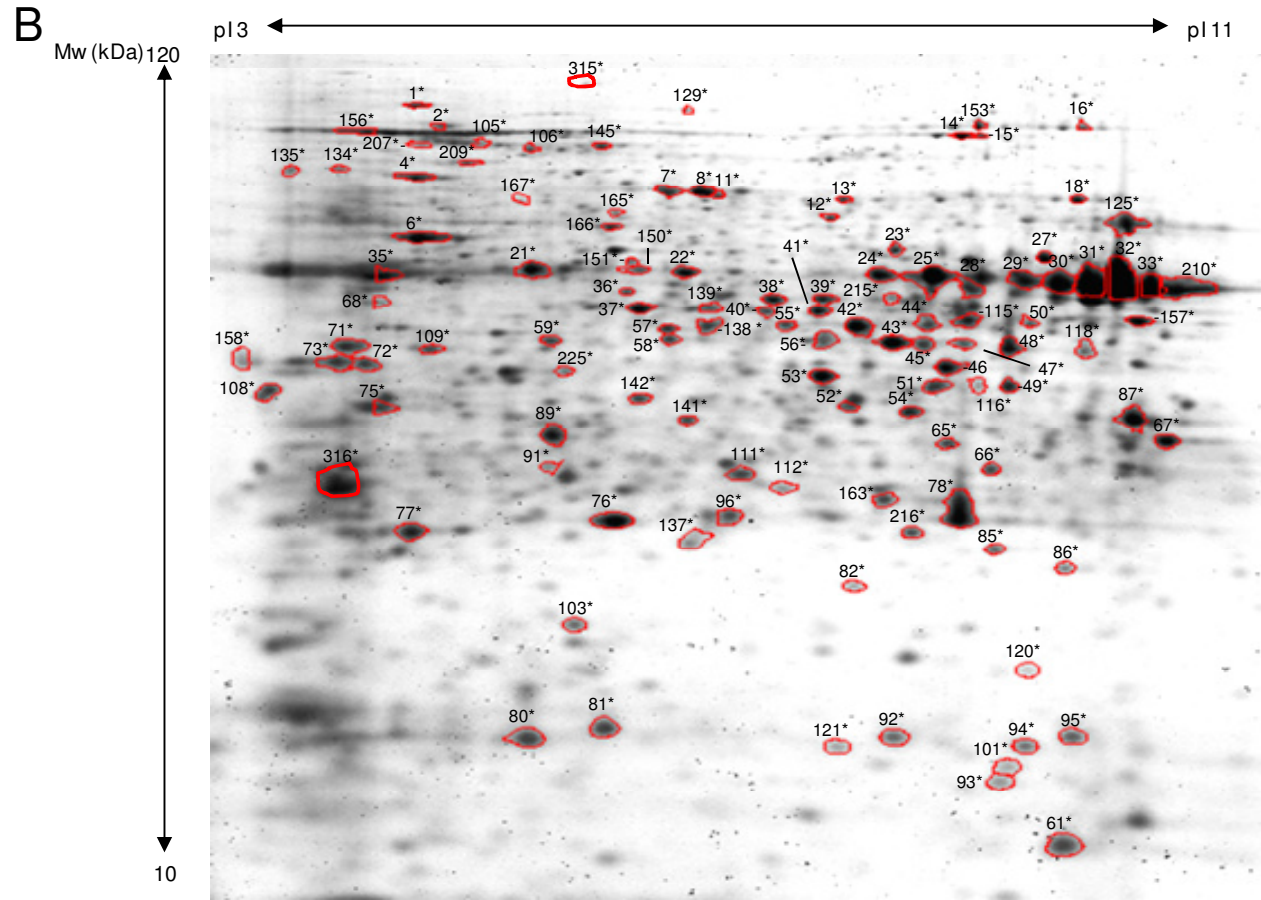


Table S2 - Change of the intracellular proteome in response to IP production during methanol induction

Samples before methanol addition at the end of the glycerol batch phase and 6, 18, 48 and 120 hours after the onset of methanol feeding were analyzed and the protein changes are shown (numbers. log₂ changes; n.a. not analyzable, color code: light orange to red, increase, light green to dark green: decrease, yellow: no significant change).

Functional category	Gene name ¹	Gene ID	0h ²	6h	18h	48h	120h
Carbohydrate metabolism							
Methanol metabolism	<i>AOX1</i>	PAS_chr4_0821	0.0	7.4	9.4	9.2	9.5
	<i>AOX1</i> (~25 kDa) ³		0.0	7.1	7.1	8.1	8.1
	<i>CTA1</i>	PAS_chr2-2_0131	0.0	1.7	2.0	1.5	2.3
	<i>DAS1</i>	PAS_chr3_0832	0.0	0.1	0.9	-0.1	-0.2
	<i>DAK</i>	PAS_chr3_0841	0.0	n.a	6.5	8.1	8.7
	<i>FLD1</i>	PAS_chr3_1028	0.0	9.4	9.9	10.7	10.8
	<i>FGH1</i>	PAS_chr3_0867	0.0	7.0	8.4	8.8	9.0
	<i>FDH1</i>	PAS_chr3_0932	0.0	10.7	11.6	12.3	12.3
	<i>FDH1</i> (~30 kDa) ³		0.0	6.2	8.3	10.1	10.5
	<i>FDH1</i> (~17 kDa) ³		0.0	0.0	8.1	9.6	10.5
	<i>FDH1</i> (~10 kDa) ³		0.0	0.0	7.1	9.3	9.4
Glycolysis	<i>GUT1</i>	PAS_chr4_0783	0.0	-0.4	0.1	-1.6	n.a
	<i>FBP1</i>	PAS_chr3_0868	0.0	0.1	0.6	0.6	0.6
	<i>TDH1</i>	PAS_chr2-1_0437	0.0	0.6	-0.3	-0.6	-1.1
	<i>FBA1</i>	PAS_chr1-1_0072	0.0	0.2	-0.6	-1.1	-1.0
	<i>PGK1</i>	PAS_chr1-4_0292	0.0	-1.0	n.a	1.1	0.4
	<i>TPI1</i>	PAS_chr3_0951	0.0	0.1	-0.4	-2.1	-2.2
	<i>GMP1</i>	PAS_chr3_0826	0.0	0.0	-0.2	-0.8	-1.1
	<i>ENO2</i>	PAS_chr3_0082	0.0	0.1	0.0	-1.1	-0.8
Ethanol metabolism	<i>ADH3</i>	PAS_chr2-1_0472	0.0	0.0	0.0	-0.7	-0.6

Functional category	Gene name ¹	Gene ID	0h ²	6h	18h	48h	120h
Citrate cycle (TCA)	<i>CIT1</i>	PAS_chr1-1_0475	0.0	1.0	0.7	0.3	0.4
	<i>ACO1</i>	PAS_chr1-3_0104	0.0	0.5	0.1	-1.0	-0.4
	<i>MDH1</i>	PAS_chr2-1_0238	0.0	0.1	-0.2	-0.1	0.5
Pentose phosphate pathway	<i>6PGD [GND]</i>	PAS_chr3_0277	0.0	0.9	0.3	-0.5	-0.9
	<i>TAL1</i>	PAS_chr2-2_0337	0.0	1.1	0.4	-1.0	-0.4
Sugar metabolism	<i>BGL2</i>	PAS_chr1-4_0426	0.0	0.2	-0.1	-1.0	-0.9
	<i>VIG9 [PSA1]</i>	PAS_chr3_0870	0.0	0.4	-0.9	-0.9	n.a
Pyruvate metabolism	<i>PDC1</i>	PAS_chr3_0188	0.0	0.0	-0.5	-1.4	-2.0
	<i>PYK2</i>	PAS_chr2-1_0769	0.0	0.6	-0.5	-2.3	-1.9
Energy metabolism							
Oxidative phosphorylation	<i>[VMA2]</i>	PAS_c131_0021	0.0	-0.1	-1.0	-1.2	-1.6
	<i>atpA</i>	PAS_chr3_0576	0.0	0.8	0.3	0.4	1.1
	<i>atpD</i>	PAS_chr2-2_0165	0.0	-0.2	0.4	-0.7	0.1
	<i>MCR1</i>	PAS_chr4_0737	0.0	0.2	0.9	0.2	1.2
Nucleotide metabolism							
	<i>YNK1</i>	PAS_chr2-2_0059	0.0	-0.2	-0.6	-1.0	-1.4
Amino acid metabolism							
	<i>ILV5</i>	PAS_chr1-1_0432	0.0	0.2	-0.1	-0.7	-0.1
	<i>metE [MET6]</i>	PAS_chr2-1_0160	0.0	0.8	-0.7	n.a	n.a
	<i>CAR2</i>	PAS_chr3_0410	0.0	0.1	-0.3	n.a	-0.3
	<i>ARO8</i>	PAS_chr1-4_0608	0.0	1.3	0.6	-0.1	0.5
	-	PAS_chr2-2_0048	0.0	0.6	-0.4	-0.7	-0.2
	<i>SAHH</i>	PAS_chr3_0890	0.0	0.0	-0.5	-1.6	-0.4
	<i>SAM2</i>	PAS_chr3_0876	0.0	0.9	-0.3	0.4	-0.1
Metabolism of cofactors and vitamins							
	-	PAS_chr4_0550	0.0	0.0	-0.7	-0.6	-1.2
	<i>THI5</i>	PAS_chr4_0065	0.0	1.5	2.3	1.0	-0.8
	<i>THI4</i>	PAS_chr3_0648	0.0	1.9	2.3	0.5	0.1

Functional category	Gene name ¹	Gene ID	0h ²	6h	18h	48h	120h
Genetic information processing							
Transcription and translation	<i>EGD2</i>	PAS_chr2-1_0755	0.0	-0.3	-0.9	-0.3	-0.2
	<i>RPP0</i>	PAS_chr1-3_0068	0.0	0.7	-0.1	-0.4	-0.2
	<i>EFB1</i>	PAS_chr2-1_0376	0.0	-0.3	-0.4	-0.9	-1.0
	<i>PST2</i>	PAS_chr1-1_0449	0.0	0.3	0.3	-0.8	-0.5
	-	PAS_chr4_0041	0.0	0.1	-0.4	-0.1	-1.0
	<i>SUB2</i>	PAS_chr1-1_0019	0.0	0.6	0.0	0.1	1.2
	<i>RPS0</i>	PAS_chr1-4_0471	0.0	-0.3	-0.4	-0.2	-2.5
	<i>CAM1</i>	PAS_chr3_1071	0.0	0.8	2.2	3.5	2.5
	<i>GCN1</i>	PAS_chr4_0327	0.0	n.a	7.4	7.1	6.5
Sorting and degradation	<i>SSC1</i>	PAS_chr3_0365	0.0	-0.3	-1.3	-2.6	-1.0
	<i>Hps90</i>	PAS_chr1-4_0130	0.0	-0.6	-1.6	-0.9	n.a
	<i>clpB [HSP104]</i>	PAS_chr1-3_0102	0.0	0.5	-1.2	-2.2	n.a
	<i>[CDC48]</i>	PAS_FragD_0026	0.0	-0.5	-1.4	-3.6	-3.4
	<i>AAP1</i>	PP7435_Chr2-0908	0.0	-0.5	-0.5	-0.8	-0.6
	<i>UBA1</i>	PAS_chr3_0856	0.0	-0.5	-1.5	n.a	n.a
	<i>PDI</i>	PAS_chr4_0844	0.0	-0.2	-1.2	-2.7	-1.7
Folding catalysts	<i>CPNA [HSP60]</i>	PAS_chr4_0158	0.0	0.0	-0.5	-1.5	-0.2
	<i>[CPR1]</i>	PAS_chr1-3_0264	0.0	-0.2	-0.7	-1.3	-1.4
	<i>HSP70-HSA1 [SSA1]</i>	PAS_chr4_0552	0.0	-0.7	-1.5	-2.9	-2.9
	<i>HSP70 [SSB]</i>	PAS_chr3_0731	0.0	0.1	-0.8	-2.5	-2.2
	<i>KAR2</i>	PAS_chr2-1_0140	0.0	0.1	-1.5	n.a	-1.4
	<i>HSP70 [SSA3]</i>	PAS_chr3_0230	0.0	-0.4	-1.0	-2.7	-2.2
	<i>HSP70 [SSA3] (~ 25 KDa)³</i>		0.0	0.3	1.4	1.8	2.4
	Cellular processes						
Transport and catabolism	<i>SOD2</i>	PAS_chr1-4_0071	0.0	0.3	0.0	-0.1	0.2
	<i>[TOM]</i>	PAS_chr4_0391	0.0	0.4	-0.1	-1.0	1.1
	<i>ACT1</i>	PAS_chr3_1169	0.0	0.1	0.2	0.0	-0.2
Cell growth and death	<i>BMH1</i>	PAS_chr2-1_0809	0.0	-0.1	0.0	-0.8	-0.9
	<i>NAP1</i>	PAS_chr2-2_0172	0.0	-0.7	-0.5	-0.4	-1.4

Functional category	Gene name ¹	Gene ID	0h ²	6h	18h	48h	120h
Others							
Stress response	<i>TSA1</i>	PAS_chr2-2_0220	0.0	-0.2	0.4	-0.1	0.6
	<i>PRX1</i>	PAS_chr1-1_0433	0.0	0.0	0.3	-0.1	0.4
	<i>TRR1</i>	PAS_chr2-2_0480	0.0	0.3	-0.1	-1.8	-1.2
	-	PAS_chr2-1_0502	0.0	0.3	-0.4	-1.4	-1.3
Unclassified	-	PAS_chr2-1_0853	0.0	0.5	0.1	-0.1	0.6
	-	PAS_chr1-1_0118	0.0	0.5	-0.1	-0.7	-0.4
	-	PAS_chr2-1_0313	0.0	-0.1	-0.3	-0.7	-0.3
	-	PAS_chr1-4_0063	0.0	1.3	0.6	0.1	-0.4
	-	PAS_FragB_0024	0.0	0.2	-0.4	-1.4	-1.2

- 1 Gene names in brackets are from *S. cerevisiae* if the names differ for both yeasts (<http://www.uniprot.org/>).
- 2 When no protein spot was detectable at the end of the glycerol batch phase (methanol metabolism), a virtual number of 0.001 was taken for the spot intensity to allow the calculation of log₂ values (spot intensities were normalized using the log₂ ratio of induced samples *versus* uninduced samples).
- 3 Fragments of full-length proteins, AOX1, FDH1 and SSA3, were identified and the respective log₂ fold changes are given

Figure S2 A and B - Changes of the intracellular proteome of *P. pastoris* X-33 in response to secretory insulin precursor with special attention to ERAD and UPR related proteins (proteome analysis of replicate cultivation)

(A) Sections of 2D gels representing parts of the intracellular proteome and containing most of ERAD and UPR related proteins at 0, 5, 16, and 72 h after induction with methanol. (B) Representative graphs of the normalized relative abundance changes of each protein spot shown in A. The spot related to fragment SSA3 ~25 kDa is shown in the small insert. Deviating from the initial feeding protocol for IP production, the methanol concentration in this cultivation was not raised stepwise but directly to 2 g L⁻¹. The small map indicates the position of the 2D sections in the entire 2D gel.

