Additional file 2: Supplementary figures for

Comparative genomics and transcriptomics of *Pichia pastoris*

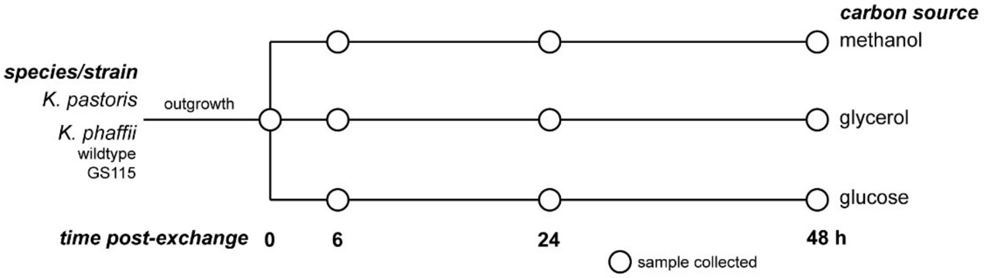


Figure S1: Experimental timeline for RNA sequencing of *Komagatella* strains. Schematic timeline for collection of RNA samples during batch cultivation of strains in shake flasks on 3 different carbon sources. Three independent cultivations were sampled for each time point.

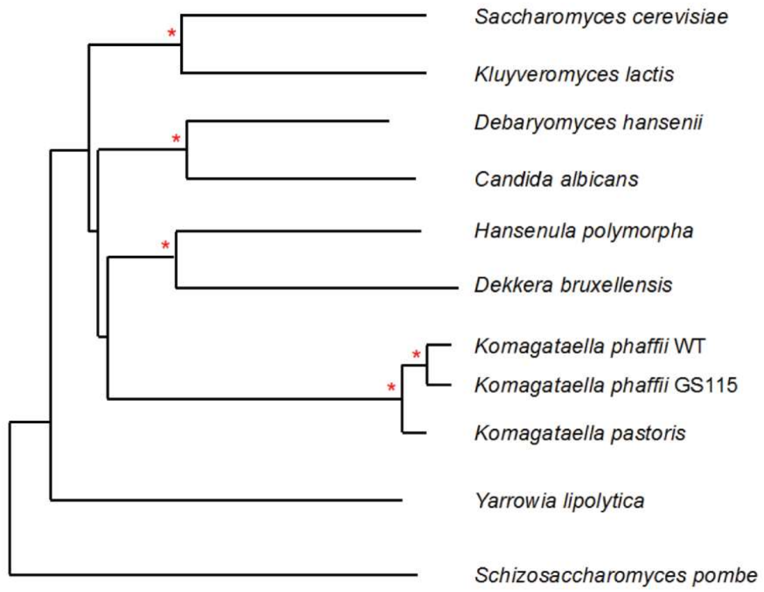


Figure S2: Phylogenetic comparison of *K. pastoris* and *K. phaffii* to other related yeasts. Phylogeny was generated using a concatenated, gap-free alignment of ten orthologous proteins. Phylogenetic tree was calculated using neighbor-joining, distance-based, maximum likelihood and maximum parsimony methods; reliability was assessed using bootstrapping. Clades marked with an asterisk are supported by 100% of bootstrap replicates in all four methods.

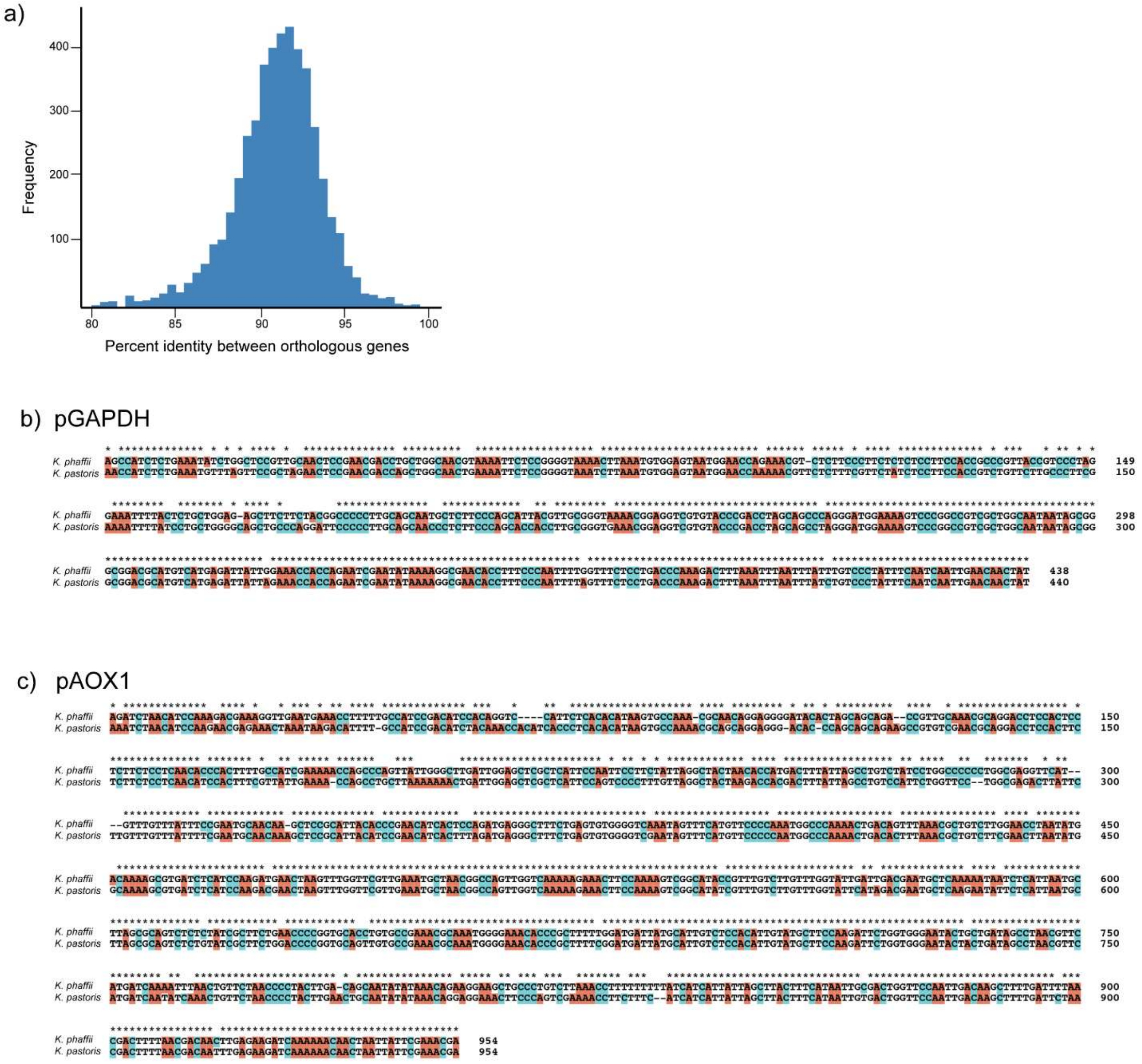
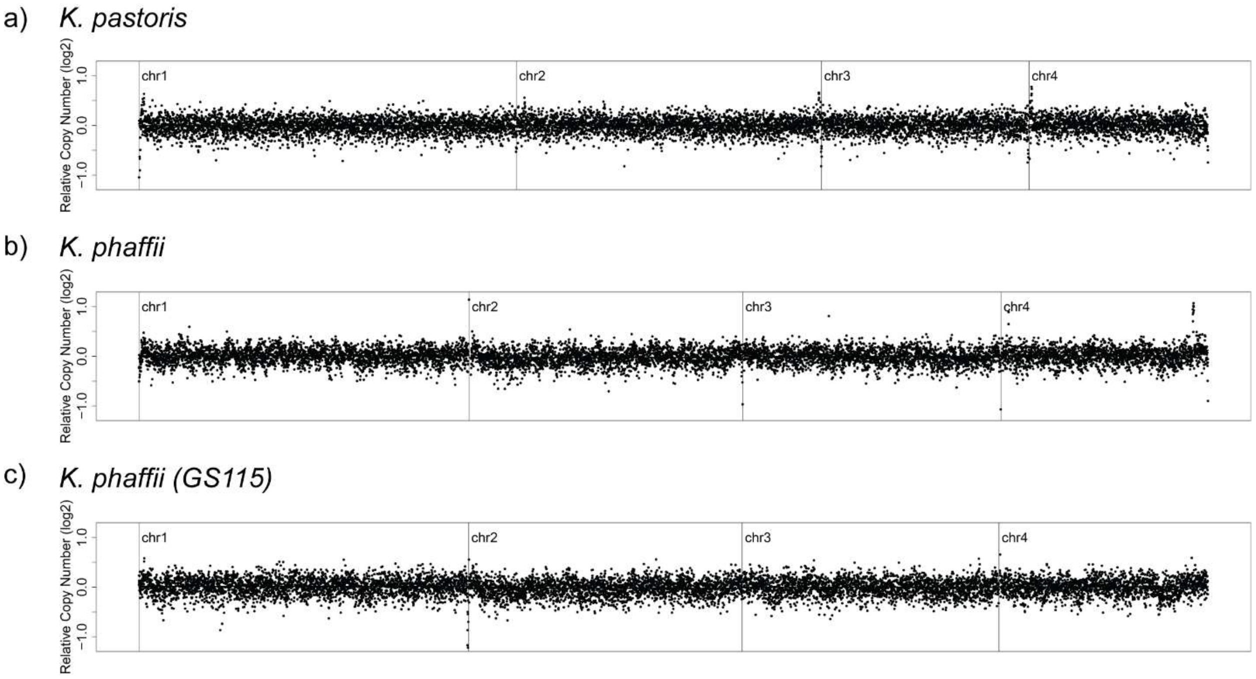


Figure S3: Gene conservation between *K. pastoris* and *K. phaffii*. a) Histogram denoting homology at the base pair level for all 1:1 orthologous genes. b) Alignment of the P_{GAPDH} promoter element between *K. pastoris* and *K. phaffii*. c) Alignment of the P_{AOX1} promoter element between *K. pastoris* and *K. phaffii*.

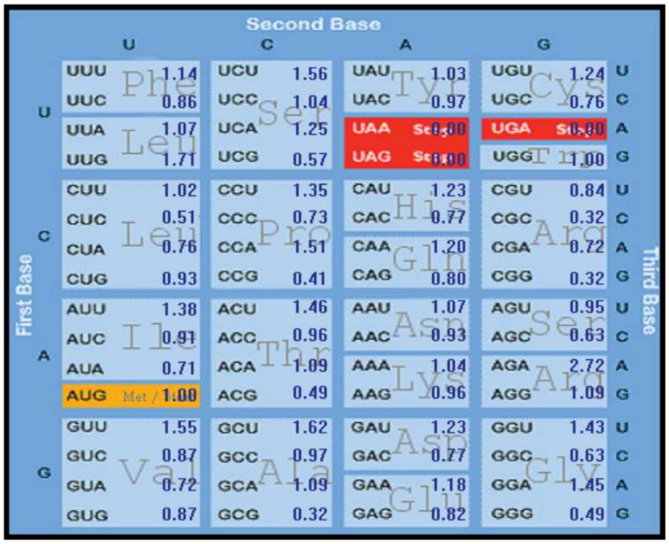

a) *K. pastoris*

b) *K. phaffii*

c) *K. phaffii (GS115)*

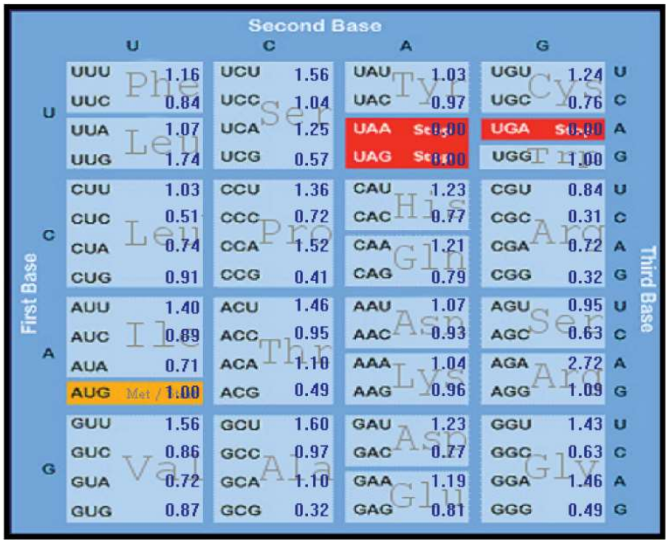
Figure S4: Copy number determination for major chromosomes in a) *K. pastoris*, b) *K. phaffii* wild-type and c) *K. phaffii* GS115 strains.

a) *K. pastoris*



	Second Base				
First Base	U	C	A	G	Third Base
U	UUU Phe 1.14	UCU Ser 1.56	UAU Tyr 1.03	UGU Cys 1.24	U
	UUC 0.86	UCC 1.04	UAC 0.97	UGC 0.76	C
	UUA Leu 1.07	UCA 1.25	UAA St 0.00	UGA St 0.00	A
	UUG 1.71	UCG 0.57	UAG St 0.00	UGG Trp 1.00	G
C	CUU Leu 1.02	CCU Pro 1.35	CAU His 1.23	CGU Arg 0.84	U
	CUC 0.51	CCC 0.73	CAC 0.77	CGC 0.32	C
	CUA 0.76	CCA 1.51	CAA Gln 1.20	CGA 0.72	A
	CUG 0.93	CCG 0.41	CAG 0.80	CGG 0.32	G
A	AUU Ile 1.38	ACU Thr 1.46	AAU Asn 1.07	AGU Ser 0.95	U
	AUC 0.91	ACC 0.96	AAC 0.93	AGC 0.63	C
	AUA 0.71	ACA 1.09	AAA Lys 1.04	AGA Arg 2.72	A
	AUG Met / 1.00	ACG 0.49	AAG 0.96	AGG 1.09	G
G	GUU Val 1.55	GCU Ala 1.62	GAU Asp 1.23	GGU Gly 1.43	U
	GUC 0.87	GCC 0.97	GAC 0.77	GGC 0.63	C
	GUA 0.72	GCA 1.09	GAA Glu 1.18	GGA 1.45	A
	GUG 0.87	GCG 0.32	GAG 0.82	GGG 0.49	G

b) *K. phaffii*



	Second Base				
First Base	U	C	A	G	Third Base
U	UUU Phe 1.16	UCU Ser 1.56	UAU Tyr 1.03	UGU Cys 1.24	U
	UUC 0.84	UCC 1.04	UAC 0.97	UGC 0.76	C
	UUA Leu 1.07	UCA 1.25	UAA St 0.00	UGA St 0.00	A
	UUG 1.74	UCG 0.57	UAG St 0.00	UGG Trp 1.00	G
C	CUU Leu 1.03	CCU Pro 1.36	CAU His 1.23	CGU Arg 0.84	U
	CUC 0.51	CCC 0.72	CAC 0.77	CGC 0.31	C
	CUA 0.74	CCA 1.52	CAA Gln 1.21	CGA 0.72	A
	CUG 0.91	CCG 0.41	CAG 0.79	CGG 0.32	G
A	AUU Ile 1.40	ACU Thr 1.46	AAU Asn 1.07	AGU Ser 0.95	U
	AUC 0.89	ACC 0.95	AAC 0.93	AGC 0.63	C
	AUA 0.71	ACA 1.10	AAA Lys 1.04	AGA Arg 2.72	A
	AUG Met / 1.00	ACG 0.49	AAG 0.96	AGG 1.09	G
G	GUU Val 1.56	GCU Ala 1.60	GAU Asp 1.23	GGU Gly 1.43	U
	GUC 0.86	GCC 0.97	GAC 0.77	GGC 0.63	C
	GUA 0.72	GCA 1.10	GAA Glu 1.19	GGA 1.46	A
	GUG 0.87	GCG 0.32	GAG 0.81	GGG 0.49	G

Figure S5: Codon usage for a) *K. pastoris* and b) *K. phaffii* as determined from all coding sequences identified in genome annotation. The relative abundance observed for each codon is represented as a percentage of total codon usage for the corresponding amino acid.

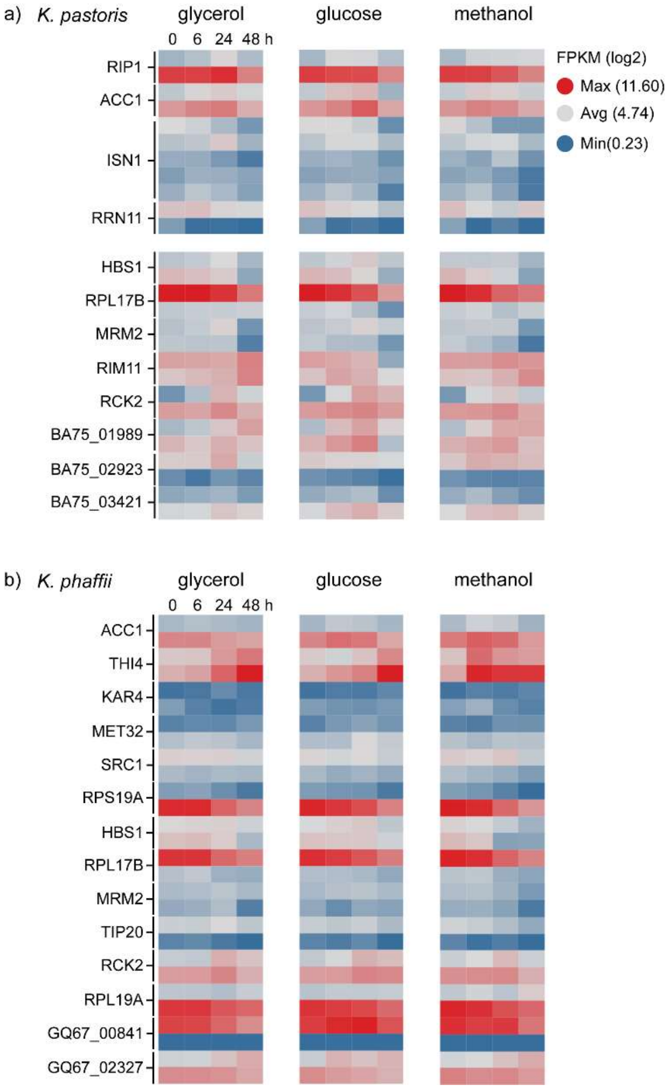


Figure S6: Isoform expression in *K. pastoris* and *K. phaffii* as a function of cultivation conditions. Heat maps of gene expression (log2 fpkm) for isoforms of alternatively spliced genes that alter coding sequences in a) *K. pastoris* and b) *K. phaffii* detected in initial genome annotation. Alternatively spliced genes with sufficient homology to *S. cerevisiae* are named, otherwise gene identifiers from genome annotation are used. Isoform expression is shown as a function of batch growth in glycerol, glucose or methanol during a 48 h cultivation period.

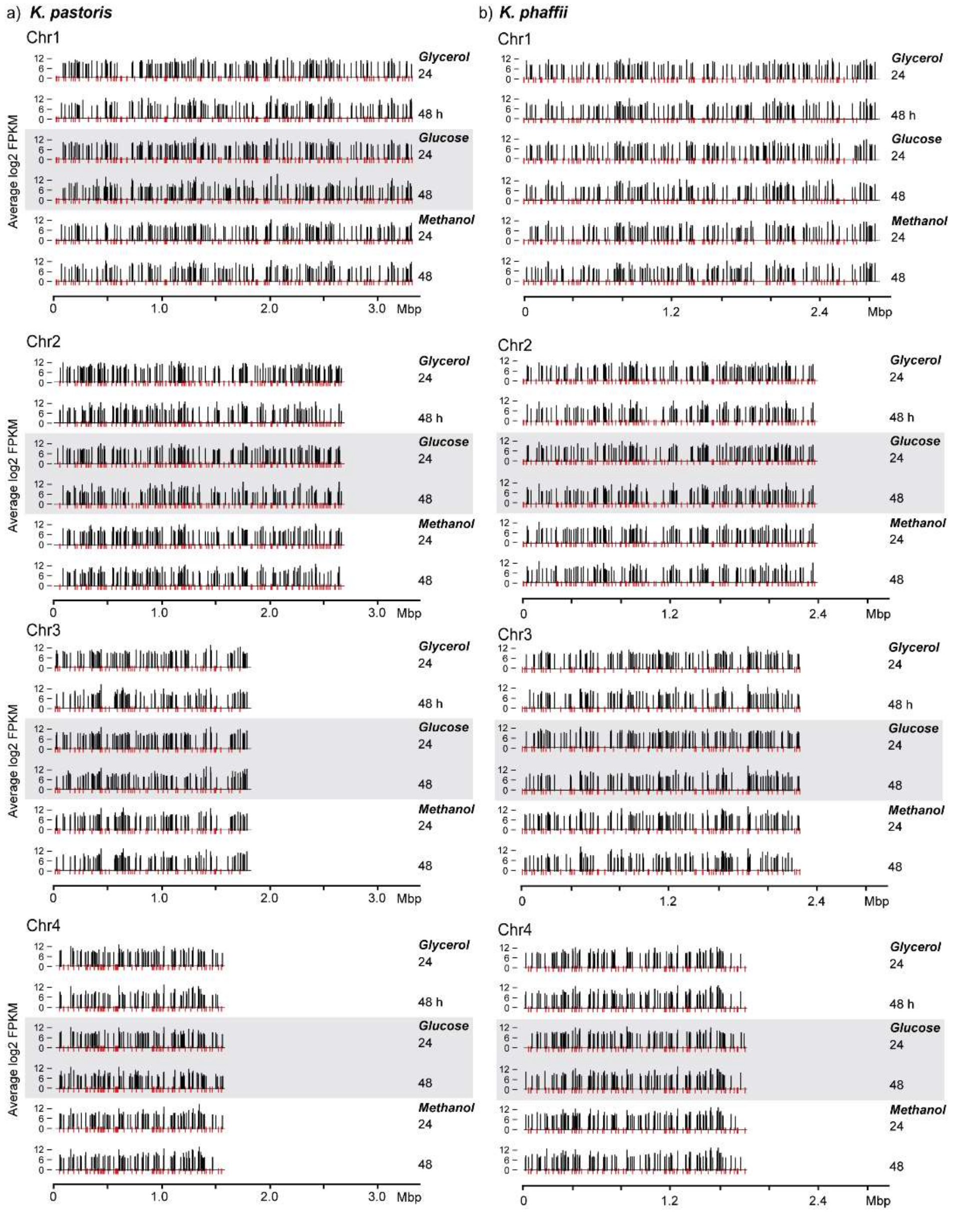


Figure S7: Chromosomal locations of highly expressed genes. Map of chromosomal location (base pair identity) for the most highly expressed genes (top 10% expression) in a) *K. pastoris* and b) *K. phaffii*. Black lines indicate gene expression level at 24 h time points during batch cultivation in either glycerol, glucose or methanol. Red lines indicate locations of GC-rich autonomously replicating sequence (GC-ARS) motifs identified by BLAST.

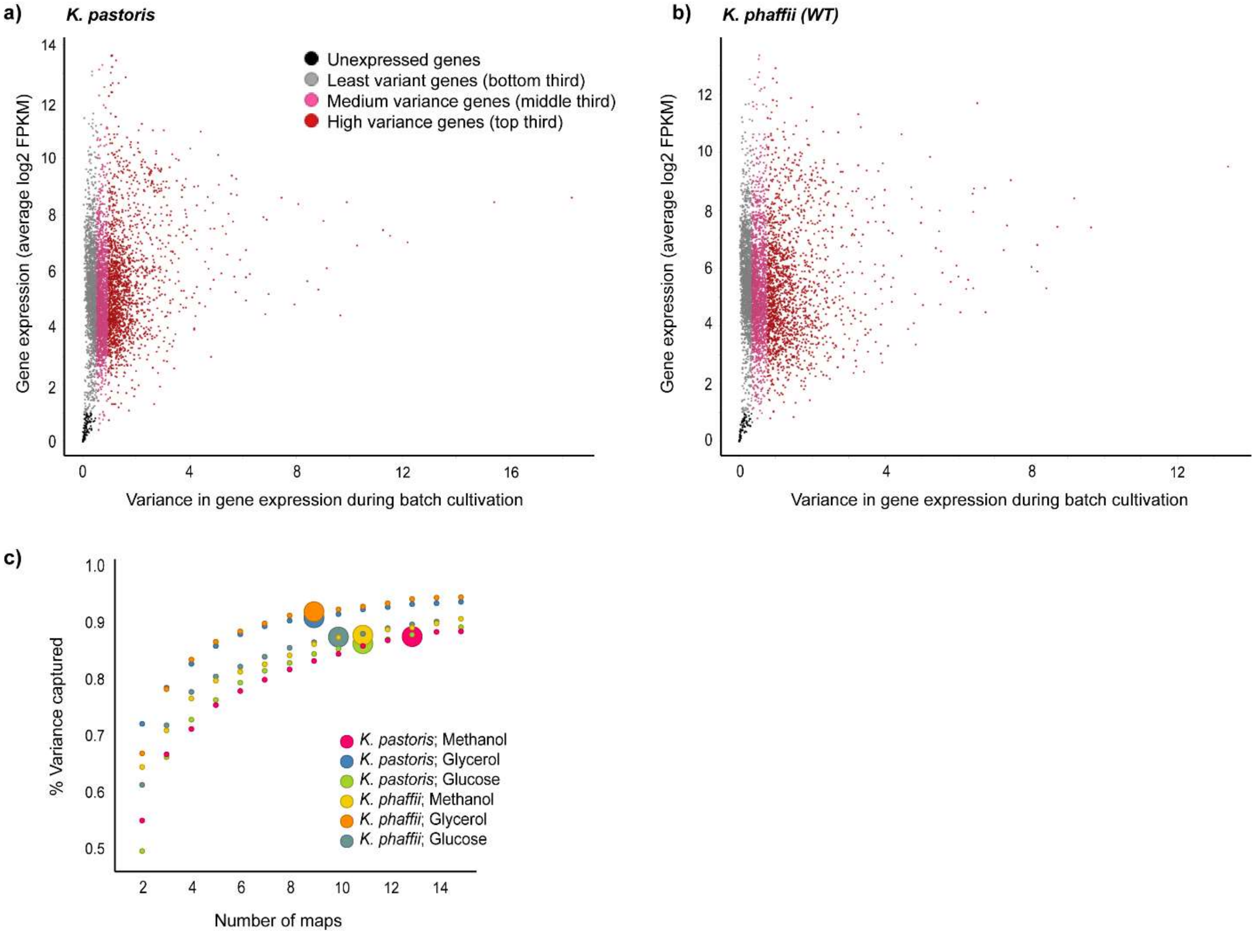


Figure S8: Variance in gene expression during batch cultivation of *K. pastoris* and *K. phaffii*. Scatter plots of the average variance versus expression observed for all annotated genes across the 10 conditional averages generated from either a) *K. pastoris* or b) *K. phaffii* expression data. Genes with average log2 fpkm <1 and variance < 0.05 were excluded from further analyses. c) Elbow analysis of input cluster number to identify optimal expression data clustering by self- organizing maps (SOMs). Large circles denote the number of clusters for each expression data set where the additional variance captured by further clustering was < 1%.

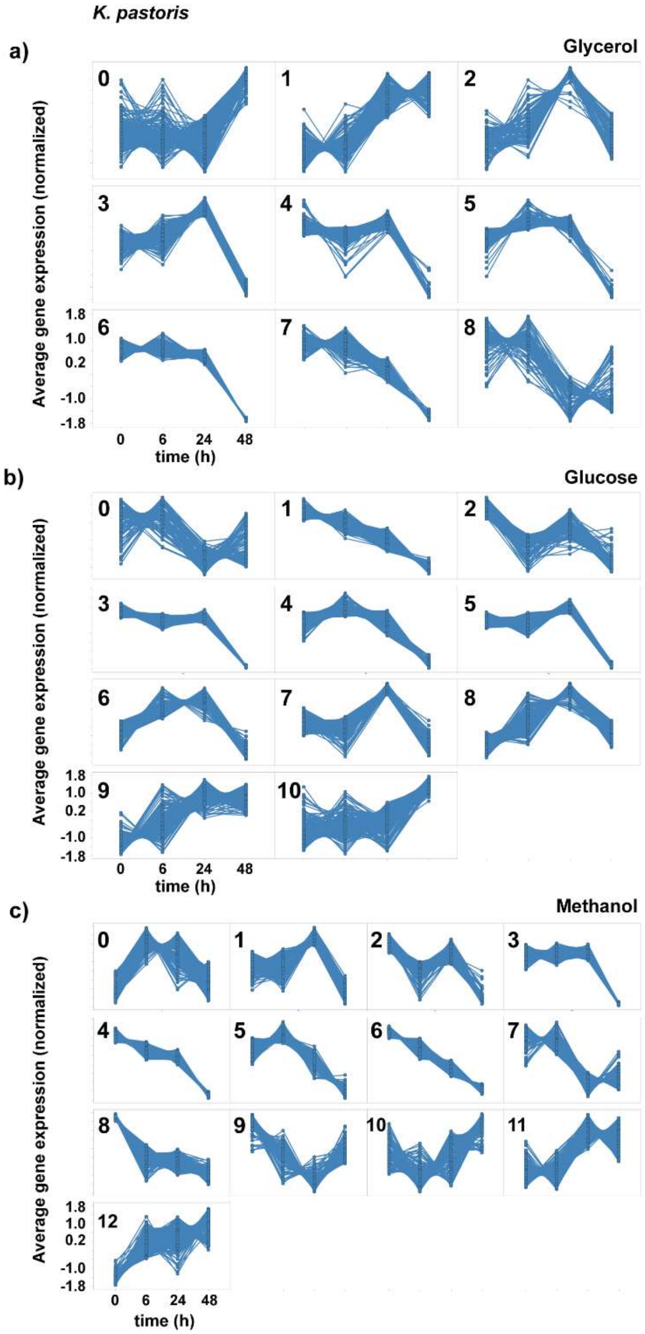


Figure S9: Gene expression phenotypes in *K. pastoris* as a function of cultivation conditions. Self-organizing maps (SOMs) of genes changing expression similarly in *K. pastoris* during a 48 h batch cultivation in a) glycerol, b) glucose or c) methanol.

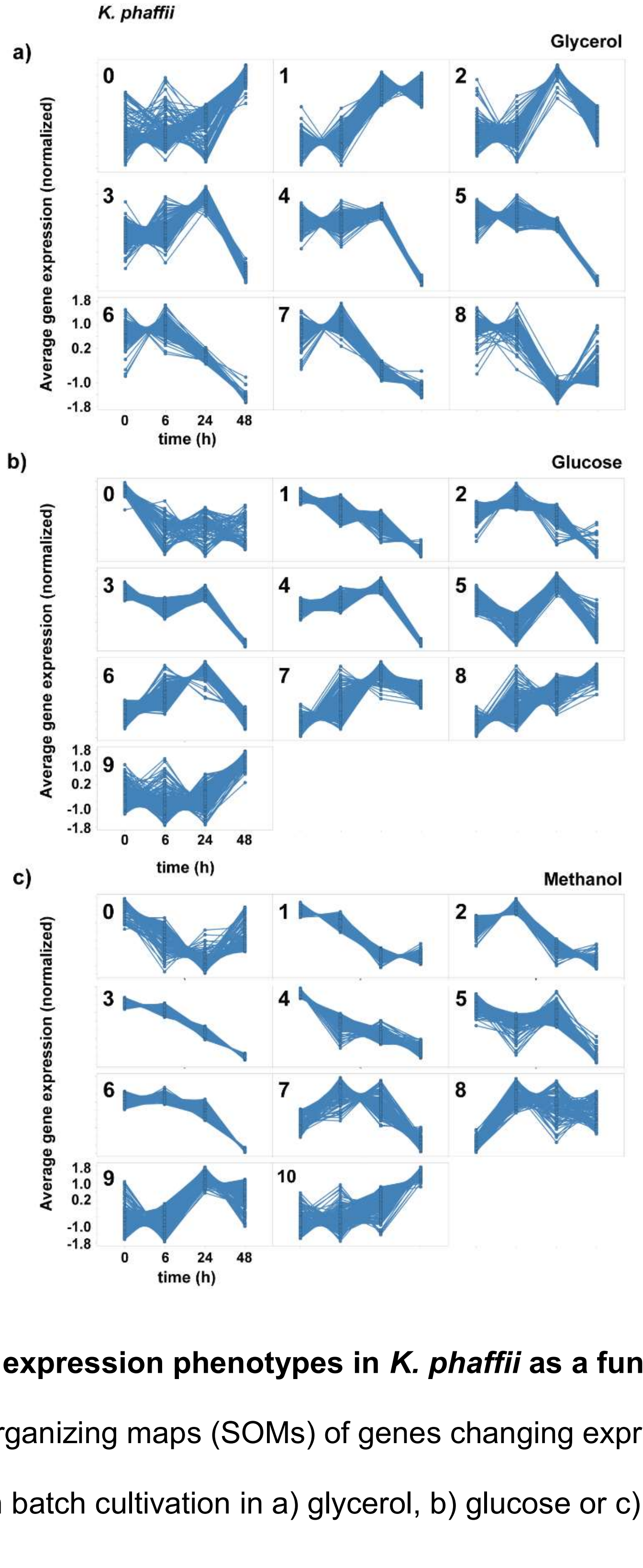


Figure S10: Gene expression phenotypes in *K. phaffii* as a function of cultivation conditions. Self-organizing maps (SOMs) of genes changing expression similarly in *K. phaffii* during a 48 h batch cultivation in a) glycerol, b) glucose or c) methanol.

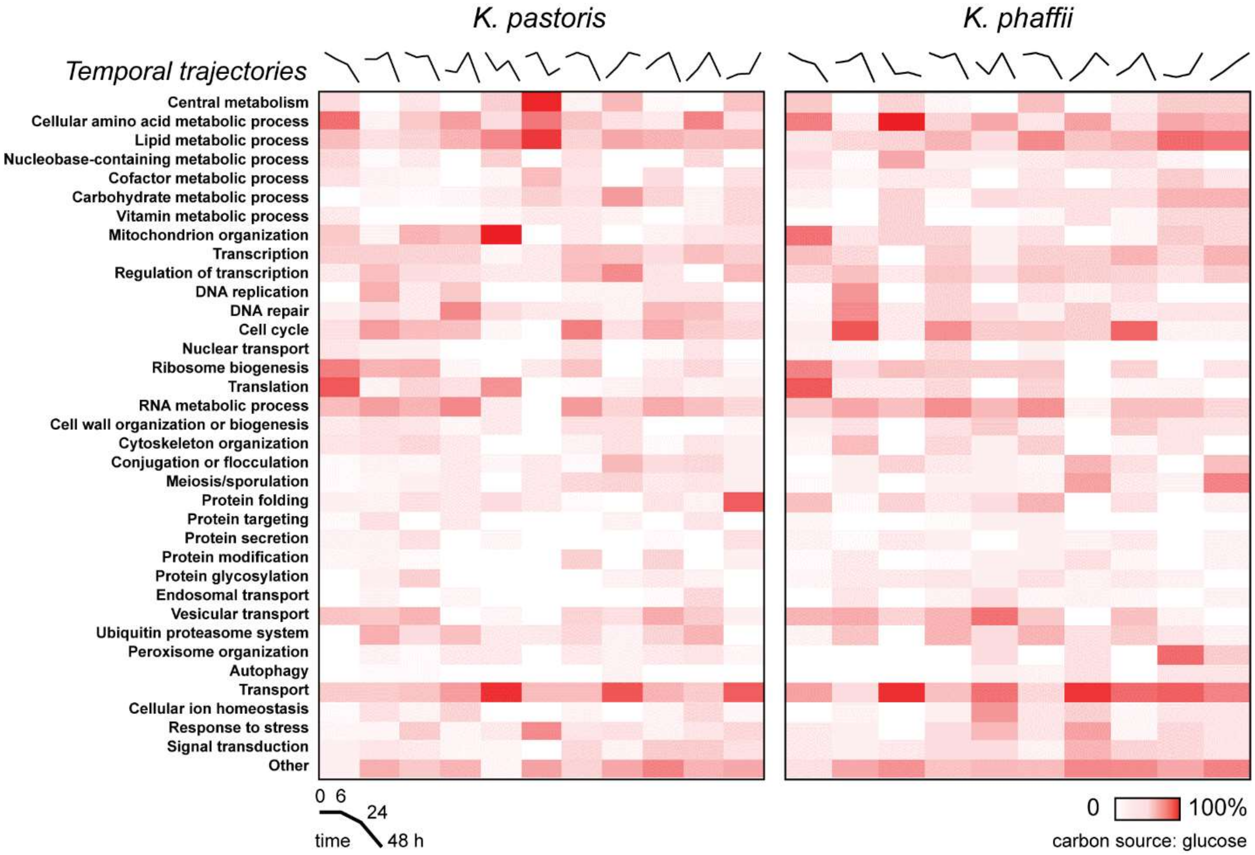


Figure S11: Biological process enrichment as a function of cultivation in glucose. Heat map representation of the enrichment of GO biological process terms for expression phenotypes observed in *K. pastoris* and *K. phaffii* during a 48 h batch cultivation in glucose as characterized by self-organizing maps (SOMs). Representative temporal trajectories of gene expression were generated for each SOM by averaging expression data at each time point for genes present within a given map. Color density relates to the number of genes assigned to a particular process as a percentage of the total number genes present in a particular expression phenotype or map.

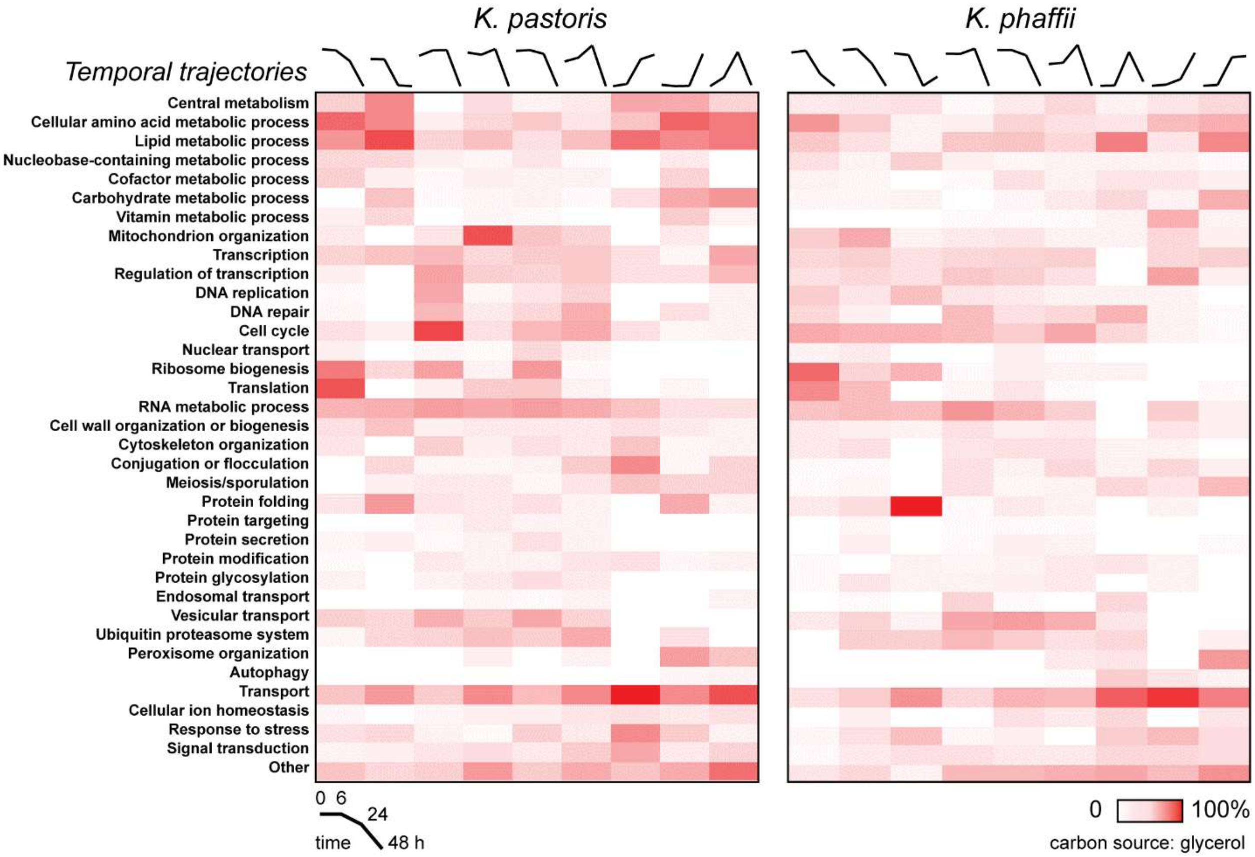


Figure S12: Biological process enrichment as a function of cultivation in glycerol. Heat map representation of the enrichment of GO biological process terms for expression phenotypes observed in *K. pastoris* and *K. phaffii* during a 48 h batch cultivation in glycerol as characterized by self-organizing maps (SOMs). Representative temporal trajectories of gene expression were generated for each SOM by averaging expression data at each time point for genes present within a given map. Color density relates to the number of genes assigned to a particular process as a percentage of the total number genes present in a particular expression phenotype or map.

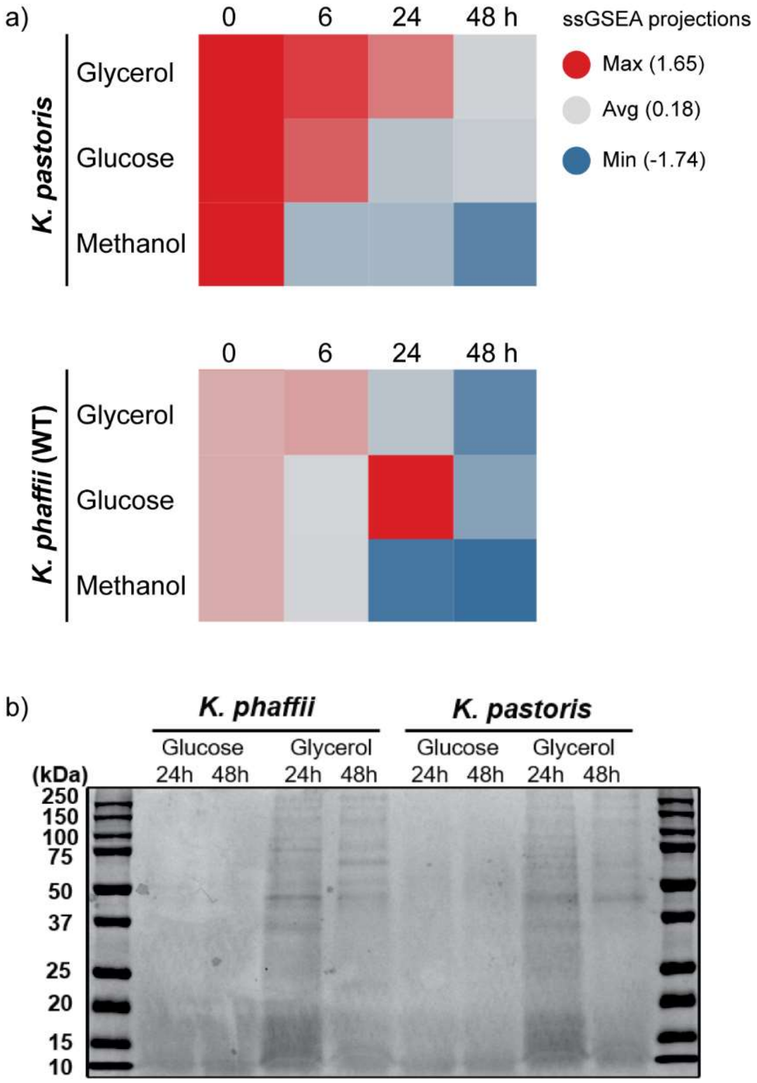


Figure S13: Secretory pathway protein expression in *K. pastoris* and *K. phaffii*.

a) Row normalized single set Gene Set Enrichment Analysis (ssGSEA) projections for 170 proteins bearing a signal peptide as identified by Signalp. b) SDS-PAGE analysis of host-cell protein expression in supernantants during batch cultivation of *K. pastoris* and *K. phaffii* for 48h in glucose or glycerol-containing media.

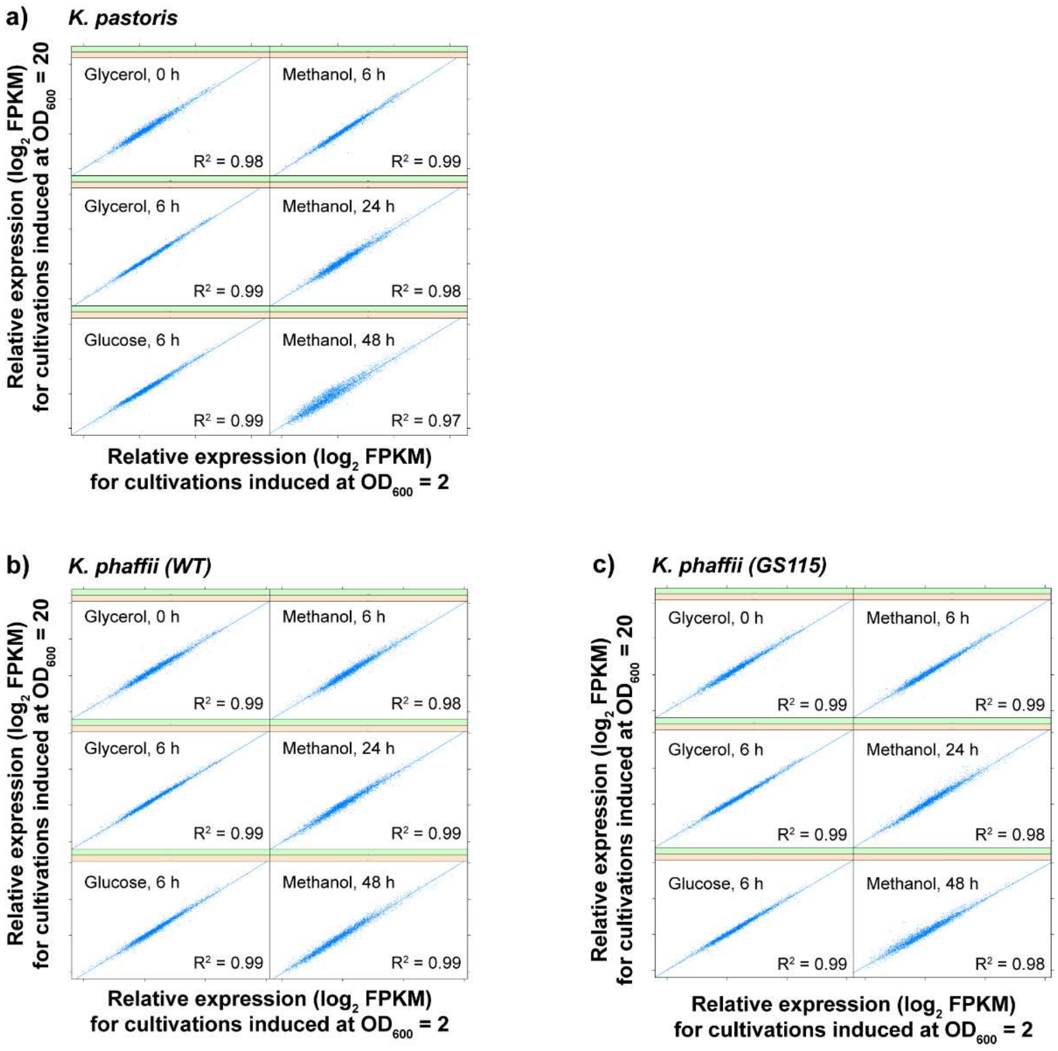


Figure S14: Correlation of gene expression at two different cultivation densities. Scatter plots of gene expression between similar cultivation conditions for a) *K. pastoris*, b) wildtype *K. phaffii*, and c) *K. phaffii* GS115 grown at two different cell densities. Density A corresponds to cultures outgrown to OD_{600} = 2.0 prior to sampling and Density B corresponds to cultures outgrown to OD_{600} = 20 prior to sampling. Pearson correlation coefficients were calculated from expression vectors that were averages of three biological replicates for each cultivation condition and density.