Supplemental Materials

Molecular Biology of the Cell

Brion et al.

Supplementary Figures

Differences in environmental stress response between yeasts is consistent with species-specific lifestyles

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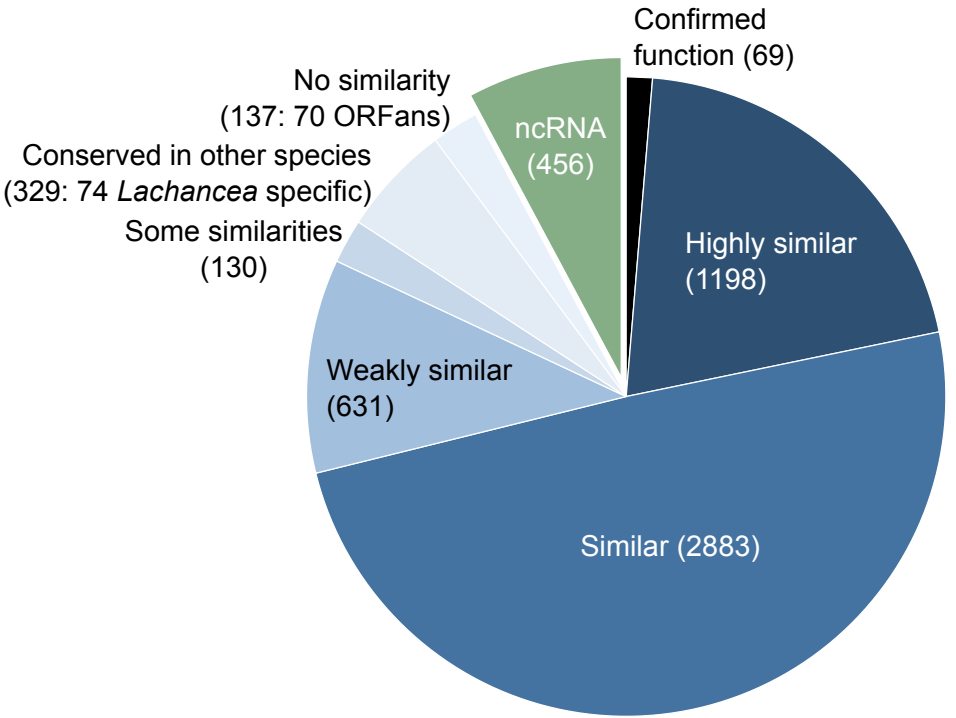
Short title: Transcriptomic stress response in yeasts

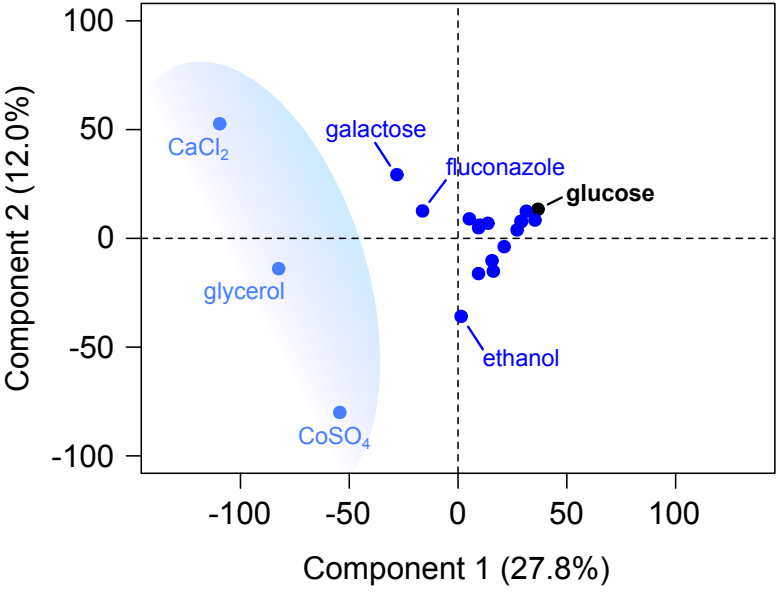
Supplementary figure 1



Overview of *L. kluyveri* gene annotation.

The annotation is based on homology to genes in *S. cerevisiae*. The percent identity for each category is as follows: highly similar: > 80%, similar: > 50%, weakly similar: > 20%, some similarities: minimum of 50% identity on at least 50 amino acids. ncRNA include pseudogenes, tRNA, rRNA and transposons. The numbers of genes are indicated in parentheses.

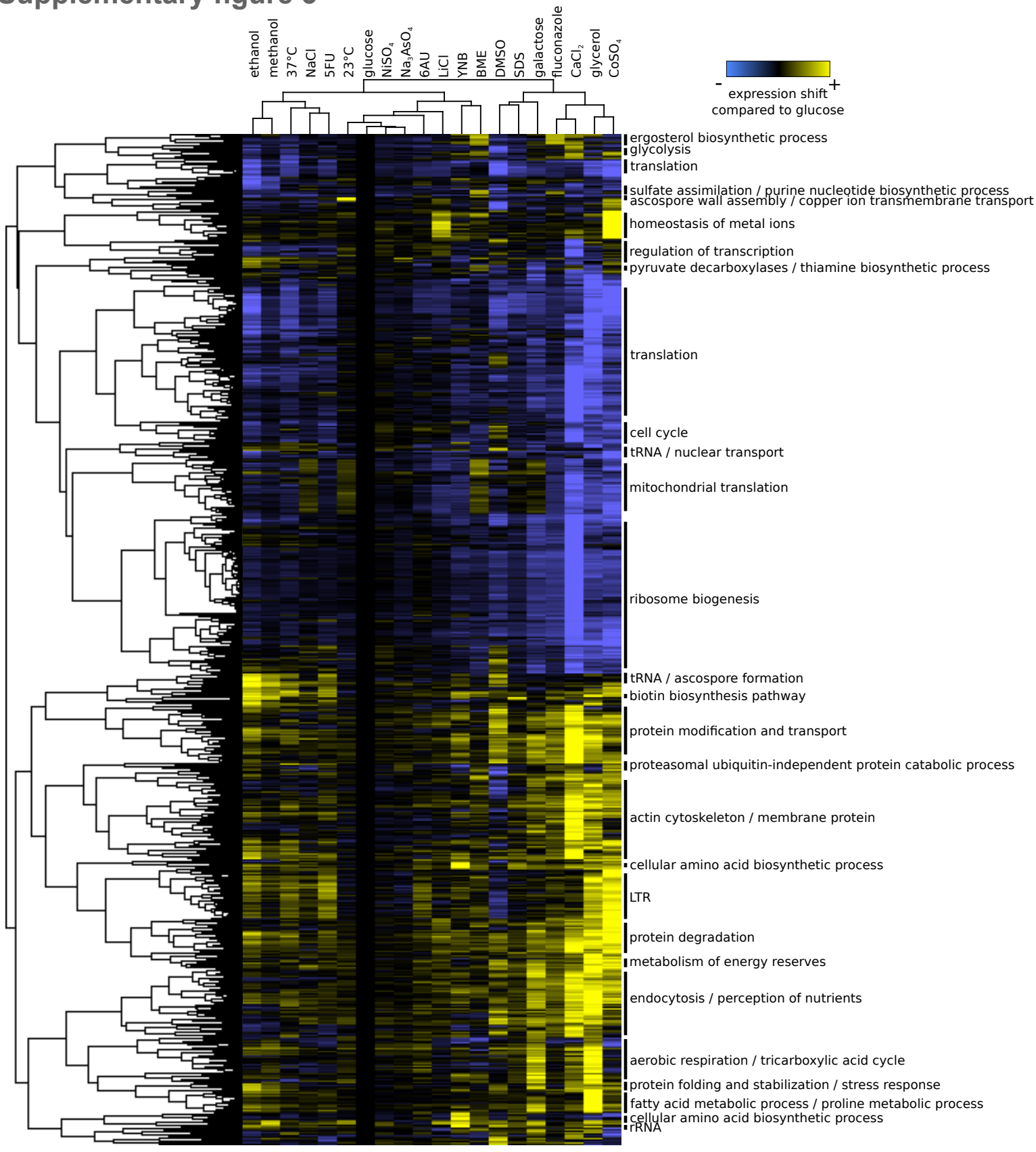
Supplementary figure 2



Principal component analysis (PCA) of the transcriptome profiles across 20 conditions.

The black dot corresponds to the non-stressful reference condition (YP-Glucose). Three conditions in particular display highly divergent profiles and are indicated in light blue.

Supplementary figure 3



Clustering analysis of expression variation for one *L. kluyveri* strain across 20 different conditions.

We used standardized $\log_2$FC values (compared to the reference medium transcriptome, *i.e.* glucose, standard deviation is corrected). Only the 2289 genes displaying significant first level clusters were used (height lower than 2.2, hclust R function). GO term enrichment analyses were performed using *S. cerevisiae* orthologs with FunSpec. 5FU: 5-fluorouracil, 6AU: 6-azauracil, BME: ß-mercaptoethanol, DMSO: dimethyl sulfoxide, SDS: sodium dodecyl sulfate.

Supplementary figure 4

		galactose	glycerol	23°C	37°C	YNB	ethanol	methanol	SDS	DMSO	NaCl	$CaCl_2$	$NiSO_4$	LiCl	$CoSO_4$	BME	5FU	arsenic	6-AU	fluconazole
	# of genes (with ortholog)	173	479	36	461	528	636	149	41	45	42	398	0	17	747	238	135	4	53	117
	# of genes (w/o ortholog)	32	119	8	164	88	198	20	12	27	25	58	0	4	172	42	52	2	24	25
respiration	TCA	++	++									+								
	electron transport chain	++	++																	
	oxidation-reduction	++	++			++	+	++	+	+		+		+	++	++		+		++
	gluconeogenesis	++																		
galactose catabolic process		++	+	+							+					+	+			
iron ion homeostasis			+	+		++								+	++	++				
transmembrane transport		+	++		++		++	++				++			++		++			+
	-drug transport				+		+	+							+	+	+			
	-ion transport		+	+										+	++					
	-amino acid transport		+				+										+		+	
sporulation	ascospore wall assembly			+	++		++	++		+							++			
	meiosis				++		++				+				+		+			
amino acid biosynthetic process						+++			+							+				
glutamate biosynthetic process			+					++												
methionine biosynthetic process																++				
proline metabolic process			+									+								+
DNA repair							++													
protein folding							+													
biotin biosynthetic process						+		+	++											+
fatty acid metabolic process		+	+			+										+			+	
ergosterol biosynthetic process																++				+++

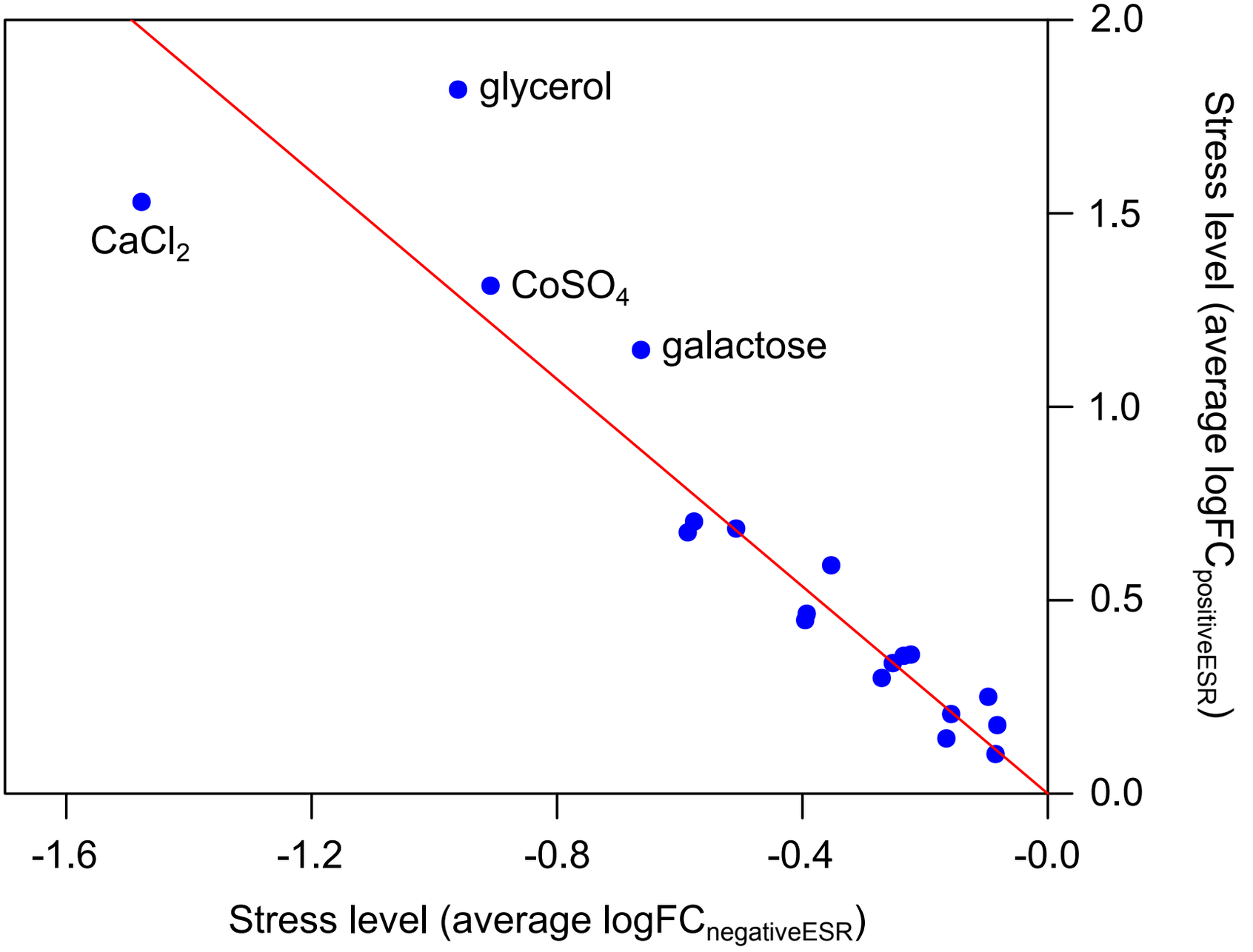
	galactose	glycerol	23°C	37°C	YNB	ethanol	methanol	SDS	DMSO	NaCl	$CaCl_2$	$NiSO_4$	LiCl	$CoSO_4$	BME	5FU	arsenic	6-AU	fluconazole
# of genes (with ortholog)	92	276	12	409	257	632	140	7	12	7	348	0	8	726	109	54	1	34	36
# of genes (w/o ortholog)	21	52	5	28	53	52	25	3	4	2	49	0	6	53	30	4	0	22	11
ATP synthesis coupled proton transport				-											--				
aerobic respiration														--					
oxidation-reduction process																			
glycolysis				-															
galactose catabolic process				-			-												
transcription		-									-								
mRNA processing											--								
mitochondrial translation											---			---					
cell cycle											-			--		-			
chromatin assembly or disassembly						-										--			
transmembrane transport	--	-	-	--	--	--	-			-				-				-	-
-amino acid transport					--								-						-
-phosphate transport					-														
amino acid biosynthetic process		--		--			-												
lysine biosynthetic process						--													
thiamine biosynthetic process	-																		
ergosterol biosynthetic process				-		-													
lipid biosynthetic process						-													
response to pheromone						--	--							-					

Funspec P-value: +++, --- : $P < 10^{-10}$; ++, -- : $P < 10^{-6}$; +, - : $P < 10^{-3}$

Table summarizing which biological processes are differently regulated under each stress condition.

The processes were determined by FunSpec GO term enrichment using the list of genes significantly up- (upper table: red) or down- (lower table: green) regulated compared to the control (YP-glucose, Benjamini Hochberg adjusted $P < 0.05$). For each condition, the numbers of genes taken into account are indicated. For the conditions highlighted in grey, no genes were significantly differentially regulated, therefore, we included genes that displayed a $\log_2$FG either greater than 1 or less than -1.

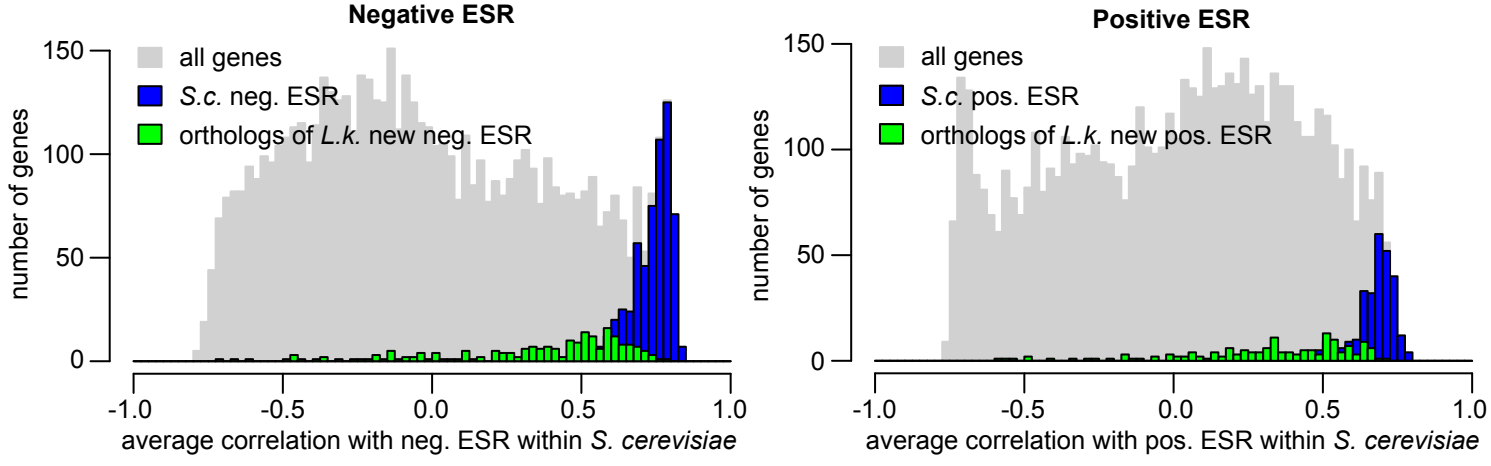
Supplementary figure 5



Comparison of the stress level estimates using negative ESR (abscissa) and positive ESR (ordinate).

A correlation of R2 = 0.94 is observed between the two measures. The conditions that present the highest stress levels are indicted.

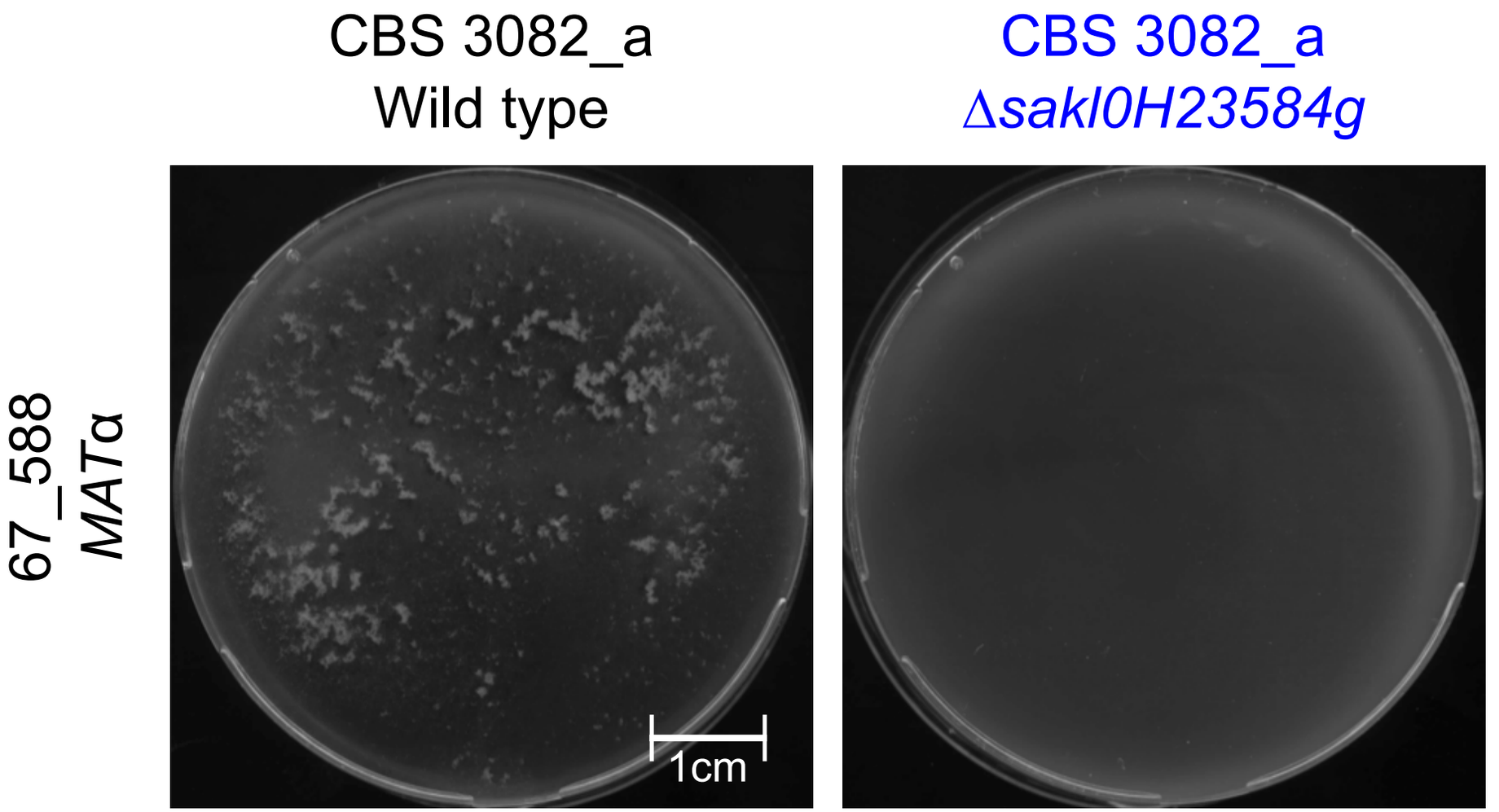
Supplementary figure 6



Study of the co-expression of the orthologs of the novel *L. kluyveri* ESR genes with *S. cerevisiae* ESR genes.

Correlations were calculated using *S. cerevisiae* expression variation across various induced stresses (data from Gasch *et al.* 2000).

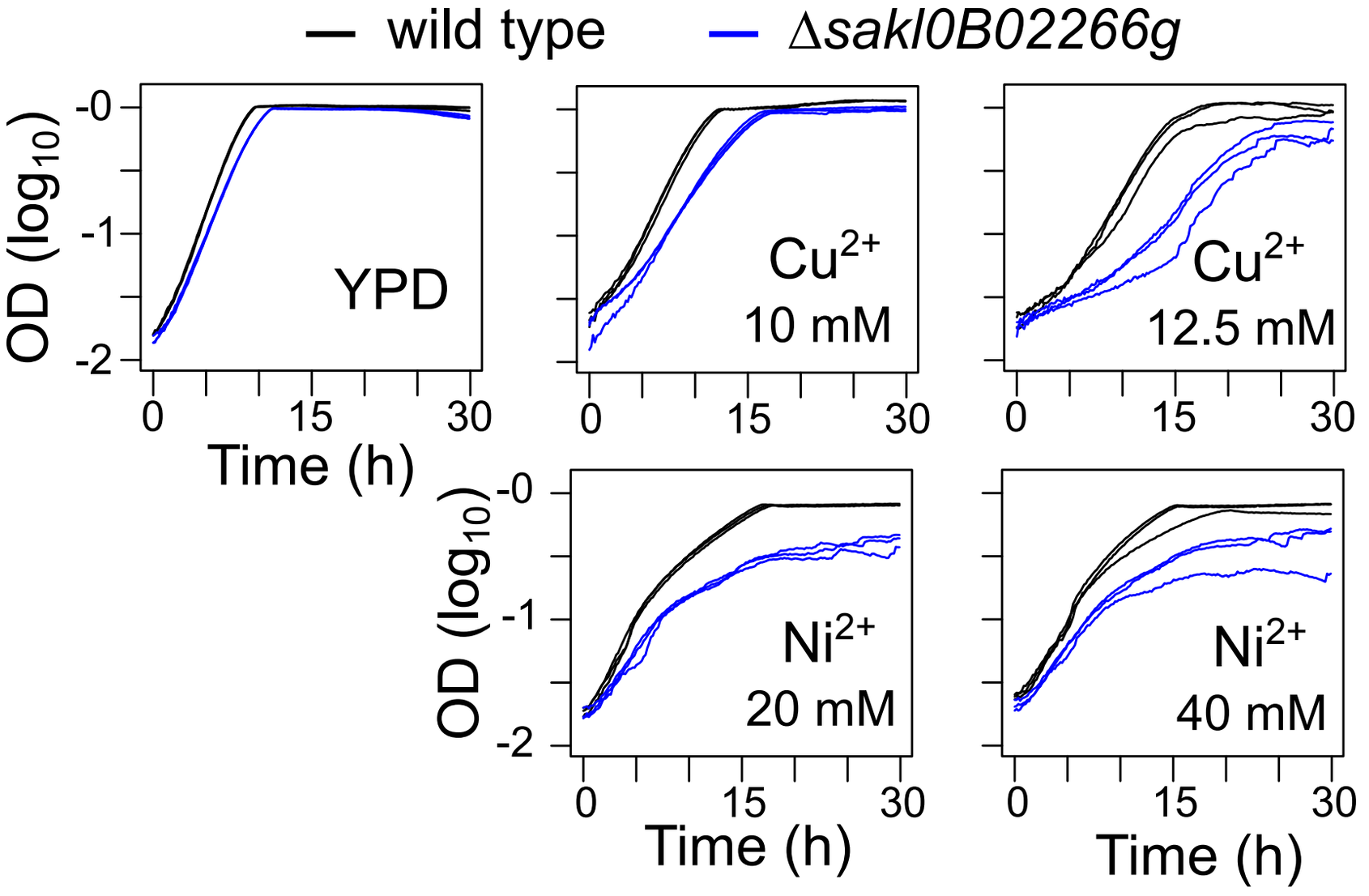
Supplementary figure 7



Effect of *SAKL0H23584g* deletion on cell agglutination during mating.

Pictures correspond to a scan of a mixture of overnight cultures of the strain 67_588 (*MAT*α) and the strain CBS 3082_a (*MAT*a) with or without the *SAKL0H23584g* gene poured in a petri dish after three hours of interaction. Significant agglutination is observed in the mix between 67_588 and CBS 3082_a wild type, while none was observed using the deletion strain at any point (up to three days after mixing the cultures).

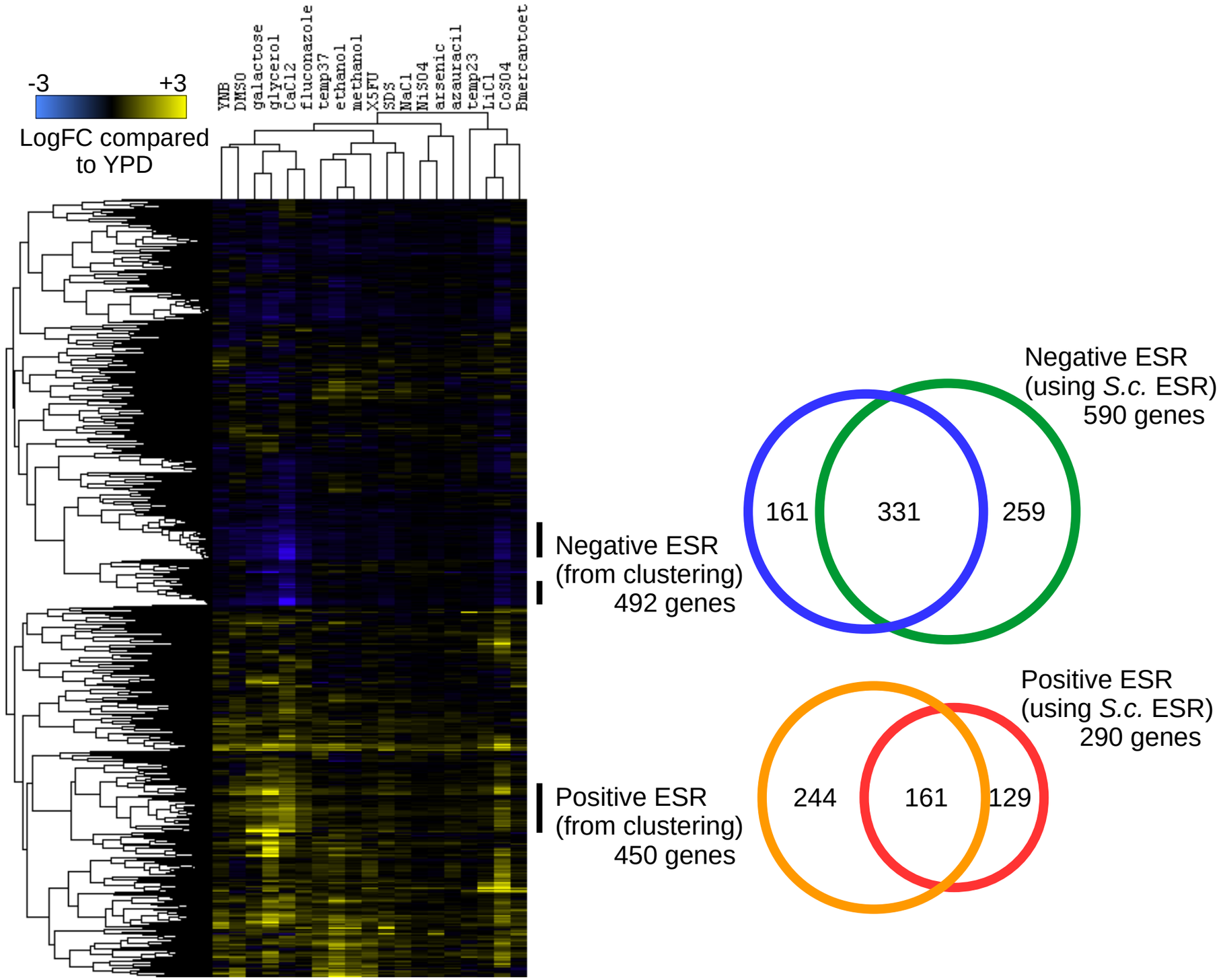
Supplementary figure 8



Functional effect of the deletion of the gene *SAKL0B02266g* potentially involved in metallic ions homeostasis.

Comparison of growth curves in the reference medium (YPD) and four different ionic stress conditions. The biomass was followed by measuring the optical density (OD) at the wave length of 600 nm using a monitoring platform TECAN F200.

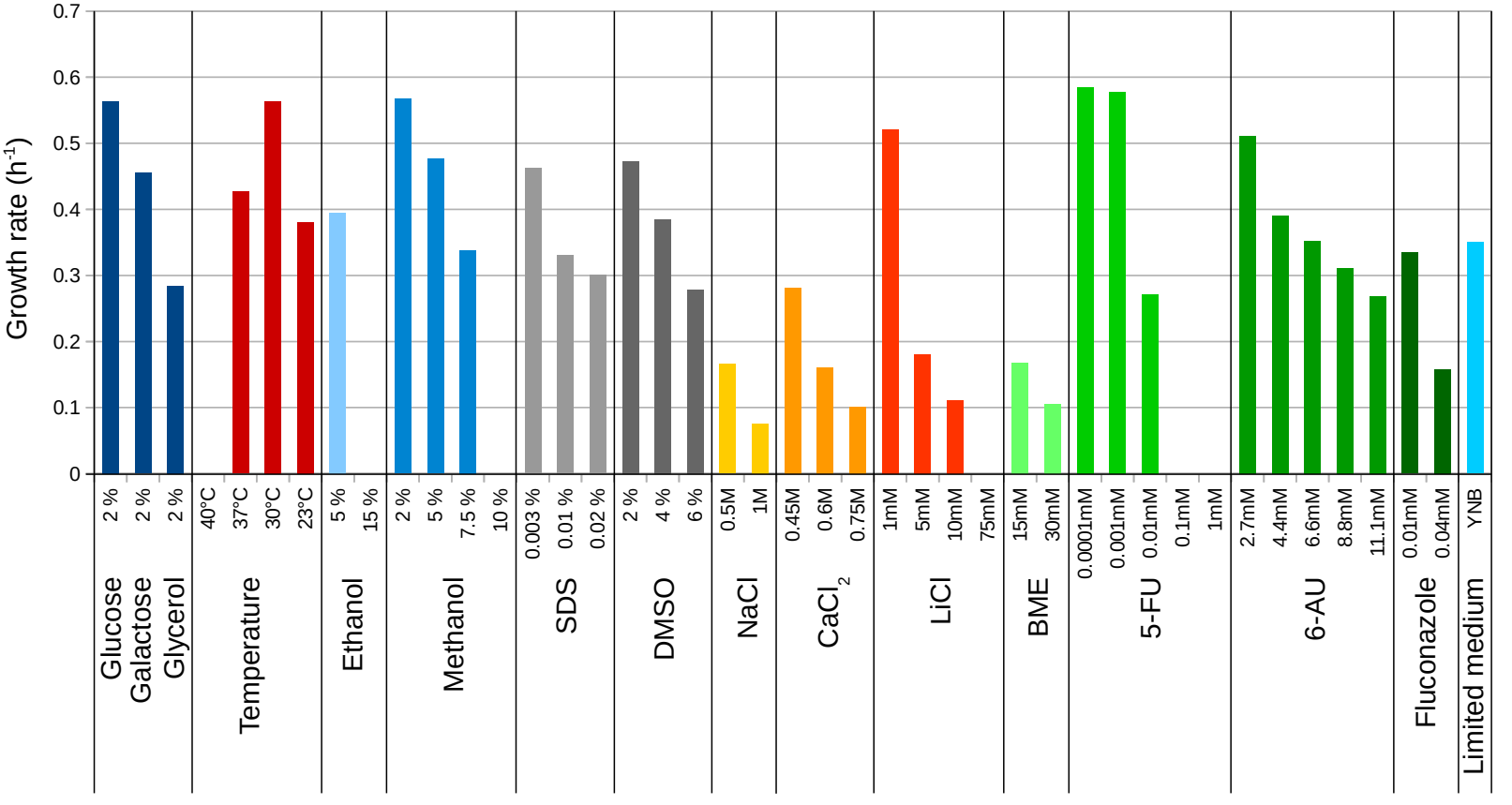
Supplementary figure 9



Determination of the ESR genes only based on the comparison between the expression profiles - stresses vs. YPD (similar approach than Gasch *et al.* 2000).

Clustering of all gene expression after normalization with the reference medium YPD (Yellow: up regulated, Blue: down regulated). The selected clusters of the ESR are the one displaying fewer up-regulation (negative ESR) or down-regulation (positive ESR). This method is compared to the ESR genes of *L. kluyveri* defined and used in the main manuscript.

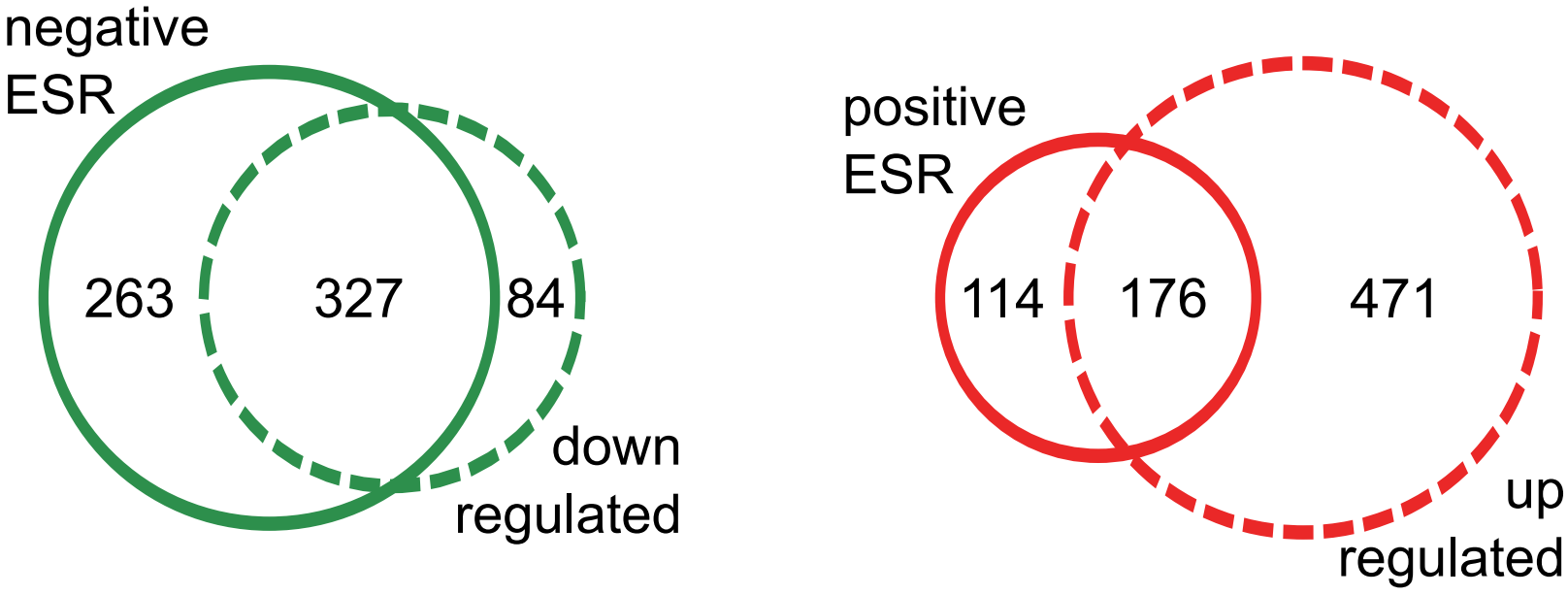
Supplementary figure 10



Dose response of growth rate of the *L. kluyveri* reference strains (CBS3092a) to various stresses.

This study helped to define the final conditions used for the mRNA profiles. Complete data in Jung *et al.* (2016). 5FU: 5-fluorouracil, 6AU: 6-azauracil, BME: ß-mercaptoethanol, DMSO: dimethyl sulfoxide, SDS: sodium dodecyl sulfate.

Supplementary figure 11



Overlap between the ESR genes and the genes with the largest changes in expression across all conditions.

The dashed circle corresponds to genes that display the largest general transcription change and were defined based on the average of the log_2FC of all conditions compared to the reference medium (YP-glucose). The average log_2FC is lower than -0.4 for the down-regulated genes and higher than 0.4 for the up-regulated genes.