Supplemental Material

- Detailed descriptions of all supplemental files -

Raw sequencing-reads. Raw sequence data (RNA-Seq data) are available on the NCBI Sequence Read Archive (SRA) under the BioProject ***PRJNA437435***. The accession numbers are listed below:

Sample	Accession
RNAseq of Y.lipolytica W29 on glucose replicate 1 1 ILLUMINA (Illumina HiSeq 4000) run: 11.2M spots, 3.4G bases, 1.3Gb downloads	SRX5799403
RNAseq of Y.lipolytica W29 on glucose replicate 2 1 ILLUMINA (Illumina HiSeq 4000) run: 14M spots, 4.2G bases, 1.6Gb downloads	SRX5799412
RNAseq of Y.lipolytica W29 on glucose replicate 3 1 ILLUMINA (Illumina HiSeq 4000) run: 1.8M spots, 531.6M bases, 213.4Mb downloads	SRX5799411
RNAseq of Y.lipolytica W29 on glycerol replicate 1 1 ILLUMINA (Illumina HiSeq 4000) run: 17.1M spots, 5.1G bases, 2Gb downloads	SRX5799410
RNAseq of Y.lipolytica W29 on glycerol replicate 2 1 ILLUMINA (Illumina HiSeq 4000) run: 24.8M spots, 7.4G bases, 2.7Gb downloads	SRX5799409
RNAseq of Y.lipolytica W29 on glycerol replicate 3 1 ILLUMINA (Illumina HiSeq 4000) run: 20.2M spots, 6.1G bases, 2.3Gb downloads	SRX5799408
RNAseq of Y.lipolytica W29 on glucose and glycerol replicate 1 1 ILLUMINA (Illumina HiSeq 4000) run: 51.7M spots, 15.5G bases, 5.7Gb downloads	SRX5799407
RNAseq of Y.lipolytica W29 on glucose and glycerol replicate 2 1 ILLUMINA (Illumina HiSeq 4000) run: 29.6M spots, 8.9G bases, 3.3Gb downloads	SRX5799406
RNAseq of Y.lipolytica W29 on glucose and glycerol replicate 3 1 ILLUMINA (Illumina HiSeq 4000) run: 47.8M spots, 14.3G bases, 5.5Gb downloads	SRX5799405
RNAseq of Y.lipolytica IBT446 on glucose replicate 1 1 ILLUMINA (Illumina HiSeq 4000) run: 37M spots, 11.1G bases, 4.5Gb downloads	SRX5799398
RNAseq of Y.lipolytica IBT446 on glucose replicate 2 1 ILLUMINA (Illumina HiSeq 4000) run: 34.7M spots, 10.4G bases, 4.1Gb downloads	SRX5799397
RNAseq of Y.lipolytica IBT446 on glucose replicate 3 1 ILLUMINA (Illumina HiSeq 4000) run: 20.4M spots, 6.1G bases, 2.3Gb downloads	SRX5799396
RNAseq of Y.lipolytica IBT446 on glycerol replicate 1 1 ILLUMINA (Illumina HiSeq 4000) run: 20.5M spots, 6.1G bases, 2.5Gb downloads	SRX5799395
RNAseq of Y.lipolytica IBT446 on glycerol replicate 2 1 ILLUMINA (Illumina HiSeq 4000) run: 17.9M spots, 5.4G bases, 2.1Gb downloads	SRX5799402
RNAseq of Y.lipolytica IBT446 on glycerol replicate 3 1 ILLUMINA (Illumina HiSeq 4000) run: 14.3M spots, 4.3G bases, 1.6Gb downloads	SRX5799401
RNAseq of Y.lipolytica IBT446 on glucose and glycerol replicate 1 1 ILLUMINA (Illumina HiSeq 4000) run: 37M spots, 11.1G bases, 4.3Gb downloads	SRX5799400
RNAseq of Y.lipolytica IBT446 on glucose and glycerol replicate 2 1 ILLUMINA (Illumina HiSeq 4000) run: 20M spots, 6G bases, 2.2Gb downloads	SRX5799399
RNAseq of Y.lipolytica IBT446 on glucose and glycerol replicate 3 1 ILLUMINA (Illumina HiSeq 4000) run: 80M spots, 24G bases, 9.7Gb downloads	SRX5799404

Table S1. Putative sugar and glycerol transporters. One of the few studies addressing sugar transport *in Y. lipolytica* was conducted by Lazar et al. (2017). The authors identified 24 putative sugar porters and tested their functionality by heterologous expression and gene deletion studies. In addition, the authors introduced the terminology *Yarrowia Hexose Transporter* (YHT 1-6) and grouped the proteins into six phylogenetic clusters (class A-F). In the present study, we investigated the expression levels of the identified transporters. Additionally, we focused on orthologs to *S. cerevisiae* aquaglyceroporin Fps1. Table S1 lists the YALI1 and YALI0 gene identifiers and provides additional information to putative transport proteins.

Table S2. Glycerol metabolic genes. Proteins involved in glycerol metabolism of *S. cerevisiae* have been used to identify corresponding proteins in *Y. lipolytica*. A blastp search (protein-protein BLAST) was conducted and the results are shown. Table S2 lists YALI1 and YALI0 gene identifiers.

File S2. Gene Ontology annotations. Gene Ontology (GO) term annotations of the *Y. lipolytica* W29 genome (GenBank assembly accession: GCA_001761485.1) were assigned by Blast2GO (Conesa et al. 2005) using the provided fungi reference database and InterProScan (Jones et al. 2014) using default settings.

File S3. Raw count values. Trimmed sequencing reads were mapped to the *Y. lipolytica* W29 reference genome (GenBank assembly accession: GCA_001761485.1) and subsequently quantified with the Subread package (Liao, Smyth, and Shi 2013) resulting in a matrix with raw count values which are provided in File S3. The count matrix is the foundation for further gene expression analyses.

File S4. TPM values. Raw read counts were converted into transcripts per million (TPM) according Wagner, Kin, and Lynch (2012), in order to compare the expression of different genes (with different gene length) across the different samples.

File S5. Gene-level statistics investigating strain and condition effect. In order to extract the strain and the condition effect we used a model describing the expression as function of strain effect (s) and carbon source condition effect (c): $\boldsymbol{y} = \boldsymbol{sx} + \boldsymbol{cx} + \boldsymbol{\epsilon}$. The strain term was categorical while the condition term was assumed to be ordinal resulting in a linear coefficient and a quadratic coefficient. File S5 provides log2 fold changes and adjusted P values for each term.

File S6. Gene-level statistics of cross comparisons. In order to analyze strain-specific responses to the applied carbon conditions, cross-comparisons between samples have been carried out. The strain and condition factors were combined into one factor and comparisons of interest were extracted as contrasts. File S6 provides log2 fold changes and adjusted P values for all contrasts.

File S7. Gene-level statistics of model 1-3. In order to investigate the influence of the different carbon sources across the two strains, we formulated three models (hypotheses) with separate factors for glucose (c_{glu}) and glycerol (c_{gly}) (present vs. not-present). In order to extract genes responding to the presence of glucose in both strains we formulated model 1: $\boldsymbol{y} = \boldsymbol{sx} + \boldsymbol{c_{glu}x} + \boldsymbol{\epsilon}$. To extract genes responding to the presence of glycerol in both strains we formulated model 2: $\boldsymbol{y} = \boldsymbol{sx} + \boldsymbol{c_{gly}x} + \boldsymbol{\epsilon}$. Finally, to extract genes differently responding in the two strains we formulated model 3: $= \boldsymbol{sx} + \boldsymbol{c_{gly,IBT}x} + \boldsymbol{c_{glu,W29}x} + \boldsymbol{\epsilon}$, where we specifically modeled the factor glycerol and IBT, and the factor W29 and glucose. File S7 provides log2 fold changes and adjusted P values for the three models (hypotheses).

Investigating the influence of glycerol on the utilization of glucose in *Yarrowia lipolytica* using RNA-Seq-based transcriptomics

Figure S1. Substrate consumption of *Y. lipolytica* strains IBT and W29 under glycerol-glucose mixed conditions. Minimal media supplemented with 5 g L^{-1} glycerol and 5 g L^{-1} glucose was used. Shake flask experiments have been conducted in duplicates.

Figure S2. Expression levels of differentially expressed genes as identified by model three. Expression levels are shown in log transcripts per million (logTPM) and names of *S. cerevisiae* orthologs are provided.