Transcriptional Response of *Chlamydomonas reinhardtii* to Small Lipid-Inducing Molecules

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1 Introduction

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1.1 Microalgae as a feedstock for biofuel production

1.2 Methods in the literature for lipid induction in microalgae

1.3 High-throughput screening for lipid-inducing small molecules

1.4 Next-generation sequencing and bioinformatics

Microalgae as a feedstock for biofuel production

- Global challenges of climate change and energy crisis
- Biodiesel: versatility and the high energy efficiency
- Microalgae: alternative feedstock for biodiesel production
- Commercial production of algal biodiesel requires higher lipid content

1 Introduction

Methods in the literature for lipid induction in microalgae

- Nutrient starvation stimulates accumulation of TAG
  - N starvation induces up to 15-fold increase in lipid bodies
  - sta6 mutant shows higher lipid accumulation during N starvation
- Often accompanied by rapid autophagic processes
  - protein recycling
  - degradation of chloroplast and ribosome
  - turnover of membrane lipids

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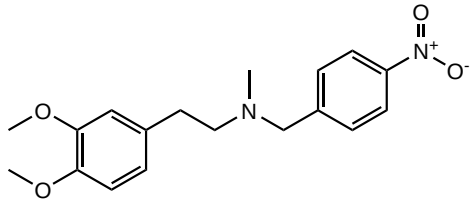
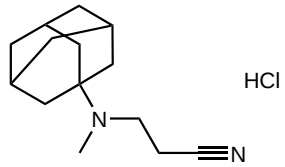
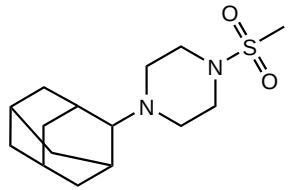
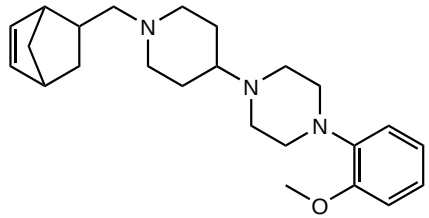
High-throughput screening for lipid-inducing small molecules

- High-throughput screening (HTS) to identify modifiers of molecular targets and cellular processes
- We identified small molecules that induce lipid accumulation with minimal impact on growth using model organism *Chlamydomonas reinhardtii*
- Four structurally diverse compounds were selected for this study

Table 1.1: Structural information of small molecules identified in HTS

ID[1]	ChemBridge ID[2]	Structure	Lab ID[3]
30	5345030		WD30030
42	5950542		WD20542
67	5234067		WD20067
84	6718784		WD10784

[1]ID of compound used in this study
[2]Unique identifier used by ChemBridge (supplier of compounds)
[3]Designation used in our lab based on chemical scaffolds

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1 Introduction

Next-generation sequencing and bioinformatics

- Next-generation RNA sequencing (RNA-seq): accurate measurement of expression and disovery of novel transcription w/o the need of hybridization
- Big data need efficient and statistically sound algorithms
- We have employed peer-reviewed tools developed by the bioinformatics community
  - TopHat/Bowtie 2: fast short reads alignment to the genome
  - Cufflinks: transcript assembly based on graph algorithm
  - eXpress: fast abundance estimation with mapping ambiguity resolution
  - GSEA: statistical information on the level of biological process

2 Targeted gene expression analysis using quantitative PCR

2 Targeted gene expression analysis using quantitative PCR

2.1 Growth and lipid accumulation

2.2 Analysis of differential gene expression

Growth and lipid accumulation

- Cells treated with compounds at a concentration of 20 µM each
- Except for compound 84, the impact on growth and photosynthesis not as severe as nitrogen starvation
- Up to 6-fold increase in lipid bodies in cells treated with compound 84
- 3- to 5-fold increase in lipid bodies in cells treated with compound 30 or 42

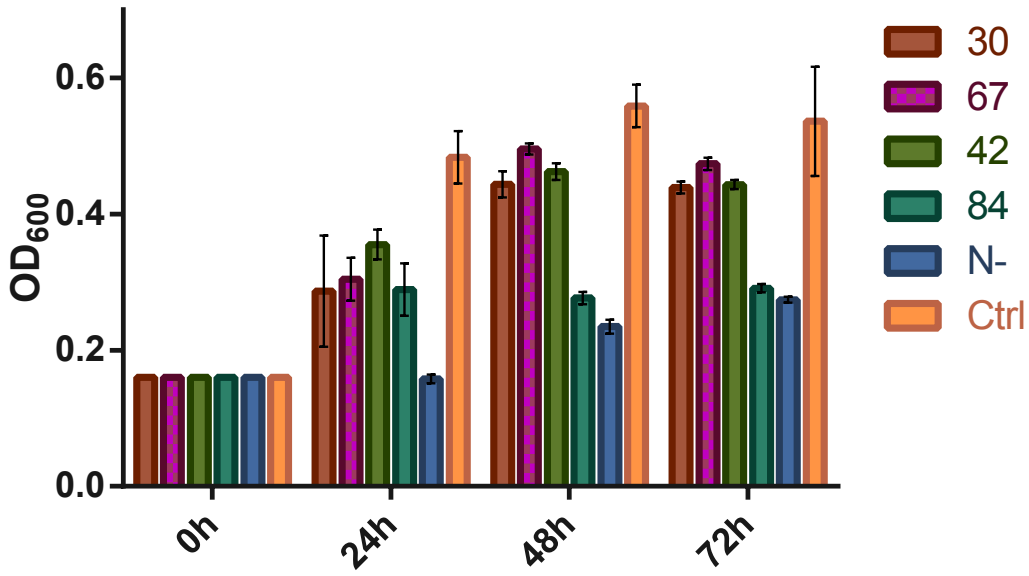


Figure 2.1: Growth under compound treatment. Cell density at 0h, 24h, 48h and 72h was estimated by optical density at 600 nm for each treatment and control. Three biological replicates were used for each condition.

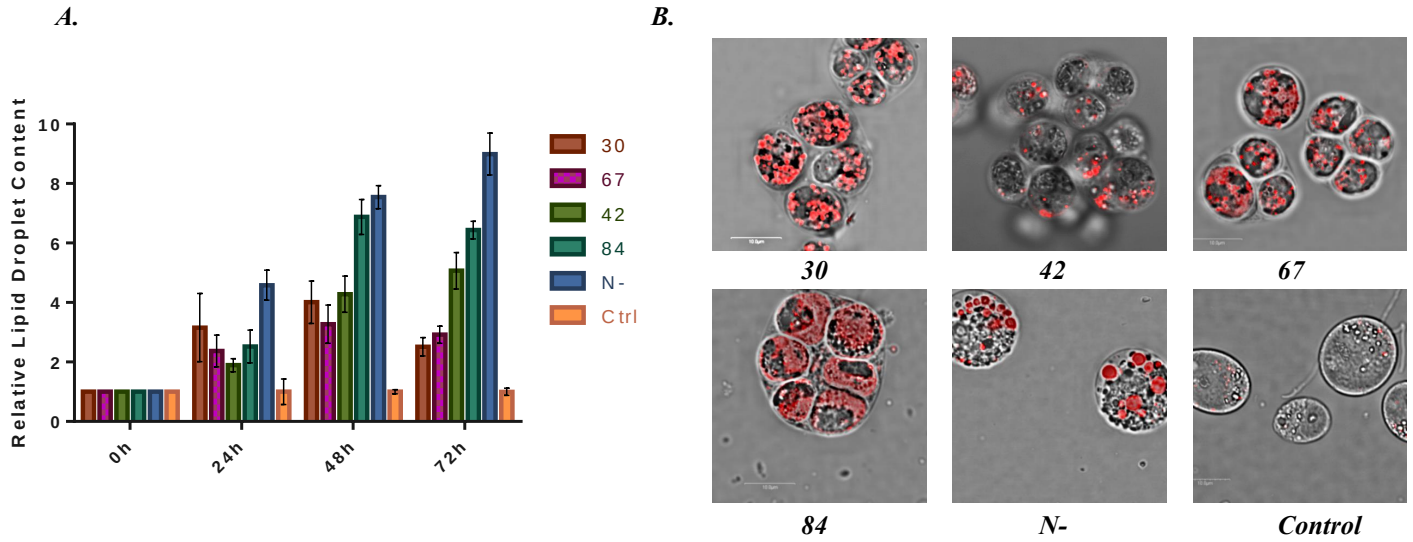


Figure 2.2: Lipid droplet accumulation during compound treatment. **A.** Lipid droplet content at 0h, 24h, 48h and 72h was estimated by fluorescence intensity after cells were stained with Nile red for each treatment and control. The fold change of fluorescence intensity relative to control was normalized by cell density in OD_{600}. Three biological replicates were used for each condition. **B.** Representative confocal microscopic images of cells stained with Nile red after 72h of incubation

2 Targeted gene expression analysis using quantitative PCR

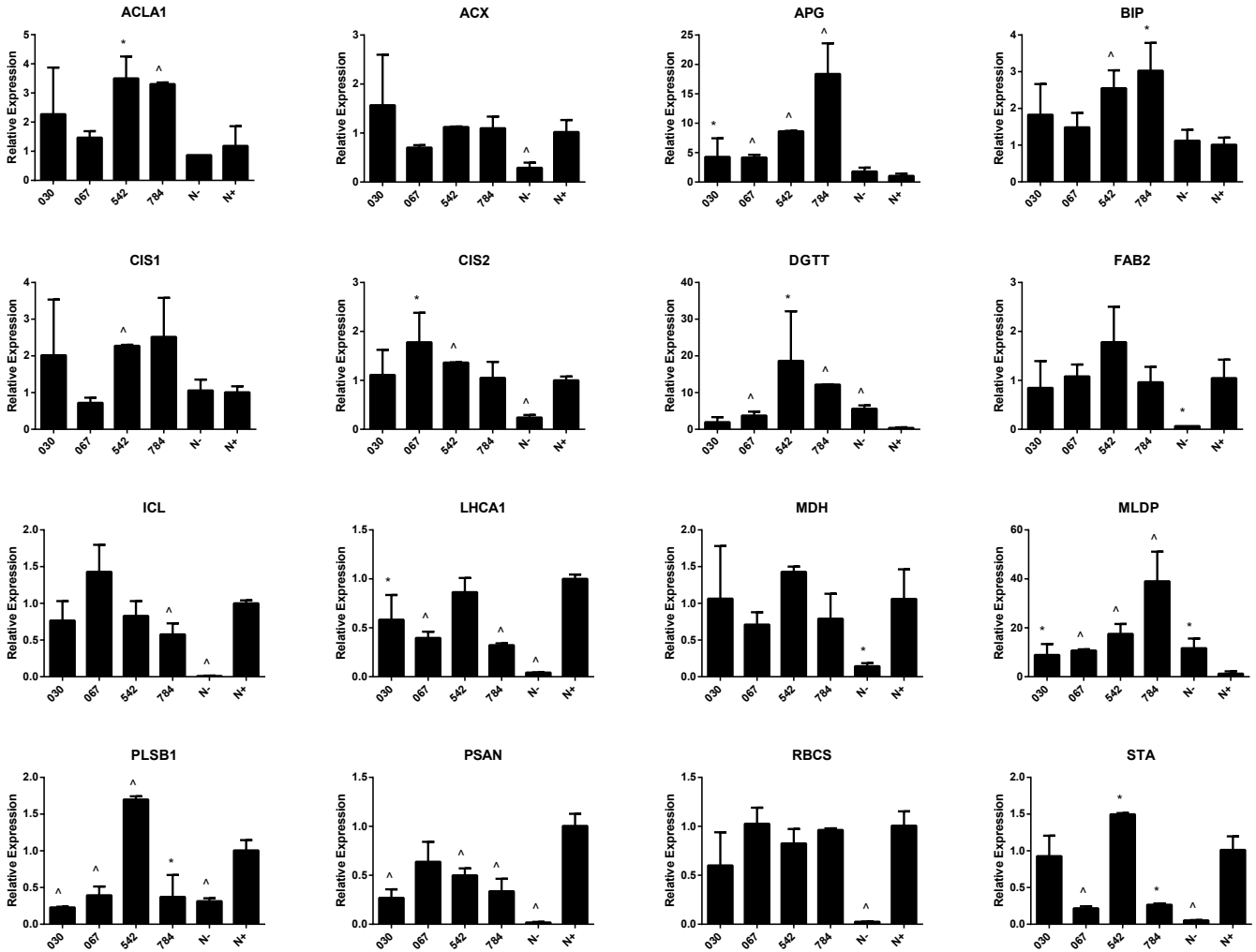
2.1 Growth and lipid accumulation

2.2 Analysis of differential gene expression

Analysis of differential gene expression

- Increased expression in DGTT and MLDP, involved in TAG biosynthesis
- Increased expression in ACL and CIS1, involved in citrate efflux from mitochondria
- Expression genes involved in photosynthesis NOT severely suppressed
- Increase expression in APG8 and BIP, involved in stress response

A.


ACLA1
ACX
APG
BIP
CIS1
CIS2
DGTT
FAB2
ICL
LHCA1
MDH
MLDP
PLSB1
PSAN
RBCS
STA
Relative Expression
030
067
542
784
N-
N+

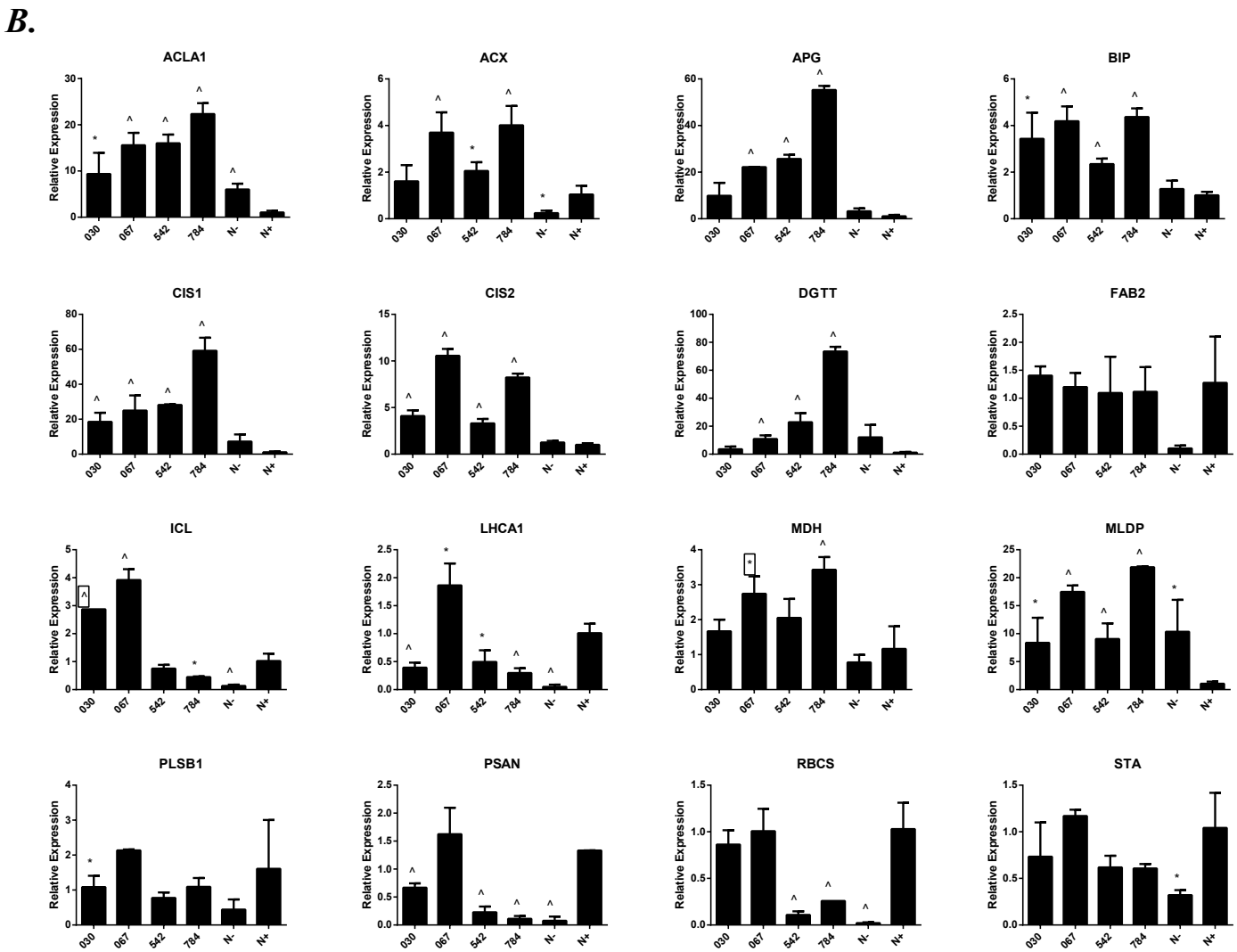


Figure 2.3: Expression of selected genes under different treatments. *: $p < 0.05$ and ^: $p < 0.01$. **A.** 24h of incubation. **B.** 72h of incubation.

3 Role of exogenous citrate in lipid production

3.1 Introduction

3.2 Growth and lipid accumulation

3.3 Uptake of citrate from the media

Introduction

- Genes involved in citrate efflux from mitochondria were significantly up-regulated
- ATP-citrate lyase catalyzes the conversion of citrate to acetyl-CoA
- Cytosolic acetyl-CoA can be used in fatty acid biosynthesis, leading to TAG accumulation

3 Role of exogenous citrate in lipid production

Growth and lipid accumulation

- Enhanced cell growth with citrate supplementation
- Up to 5-fold increase in lipid bodies with 5 mM citrate
- FAMEs assay showed 1.5-fold increase in total FA and distinct FA composition

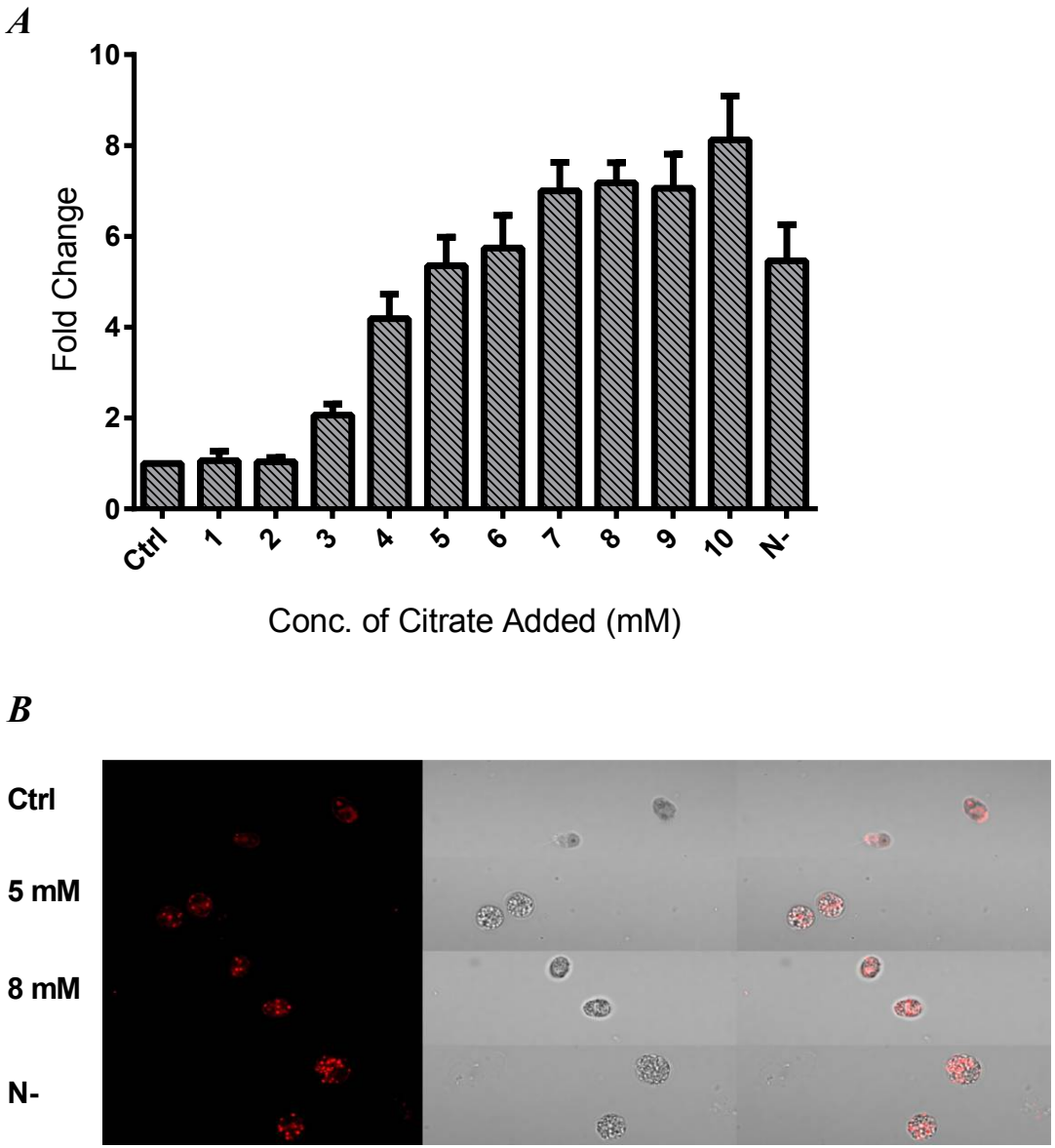


Figure 3.1: Lipid droplets accumulation during citrate supplementation. **A.** Normalized Nile red fluorescence. **B.** Confocal image at 72h of incubation.

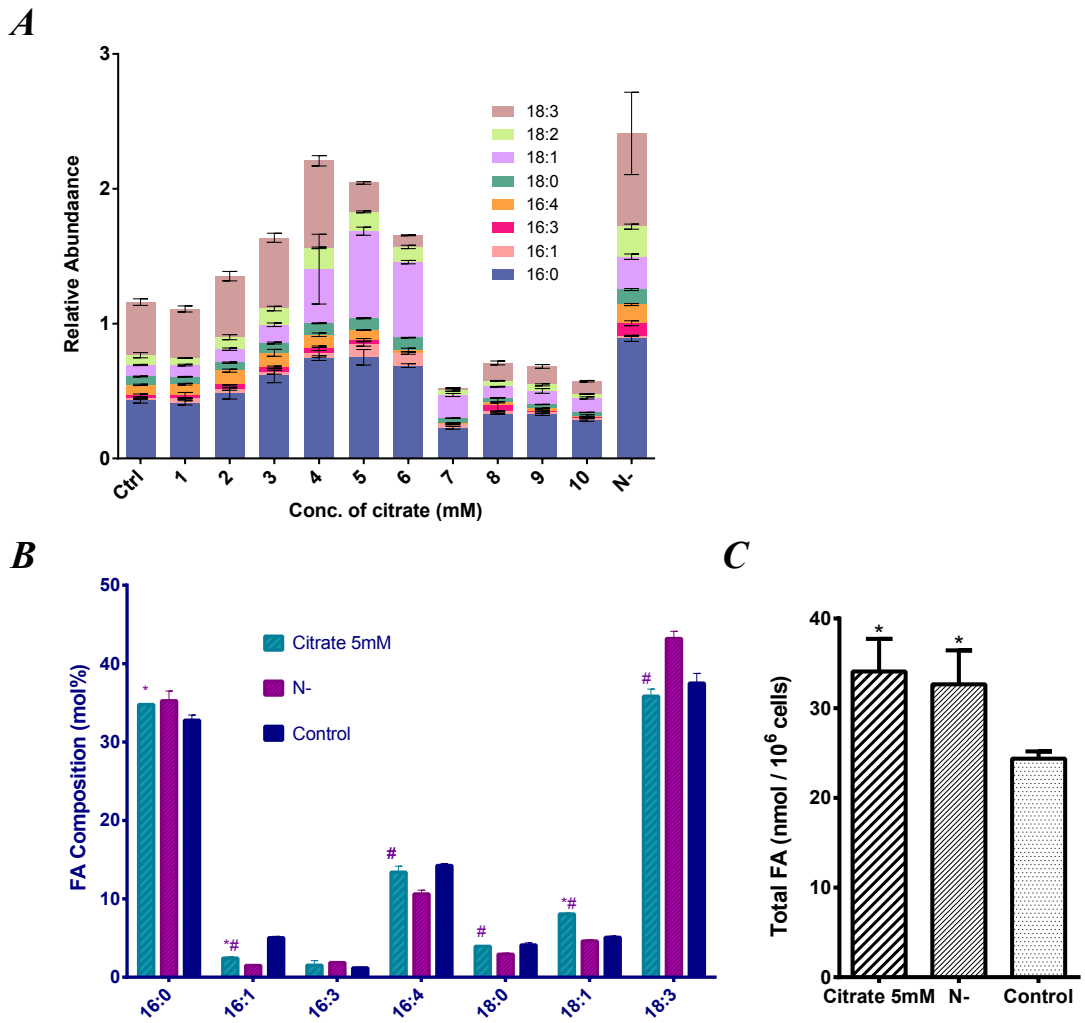


Figure 3.2: FAMEs profiles during citrate supplementation. **A.** Relative abundance of FA species at different citrate concentration. **B.** FA composition after 72h of incubation. **C.** Total FA after 72h of incubation, normalized to cell count.

3 Role of exogenous citrate in lipid production

3.1 Introduction

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3.3 Uptake of citrate from the media

Uptake of citrate from the media

- GC/MS was used to determine the amount of citrate in the cells and media
- 30-fold increase of citrate in the cells after 24h treatment with 5 mM citrate
- Citrate concentration in the media decreased over time

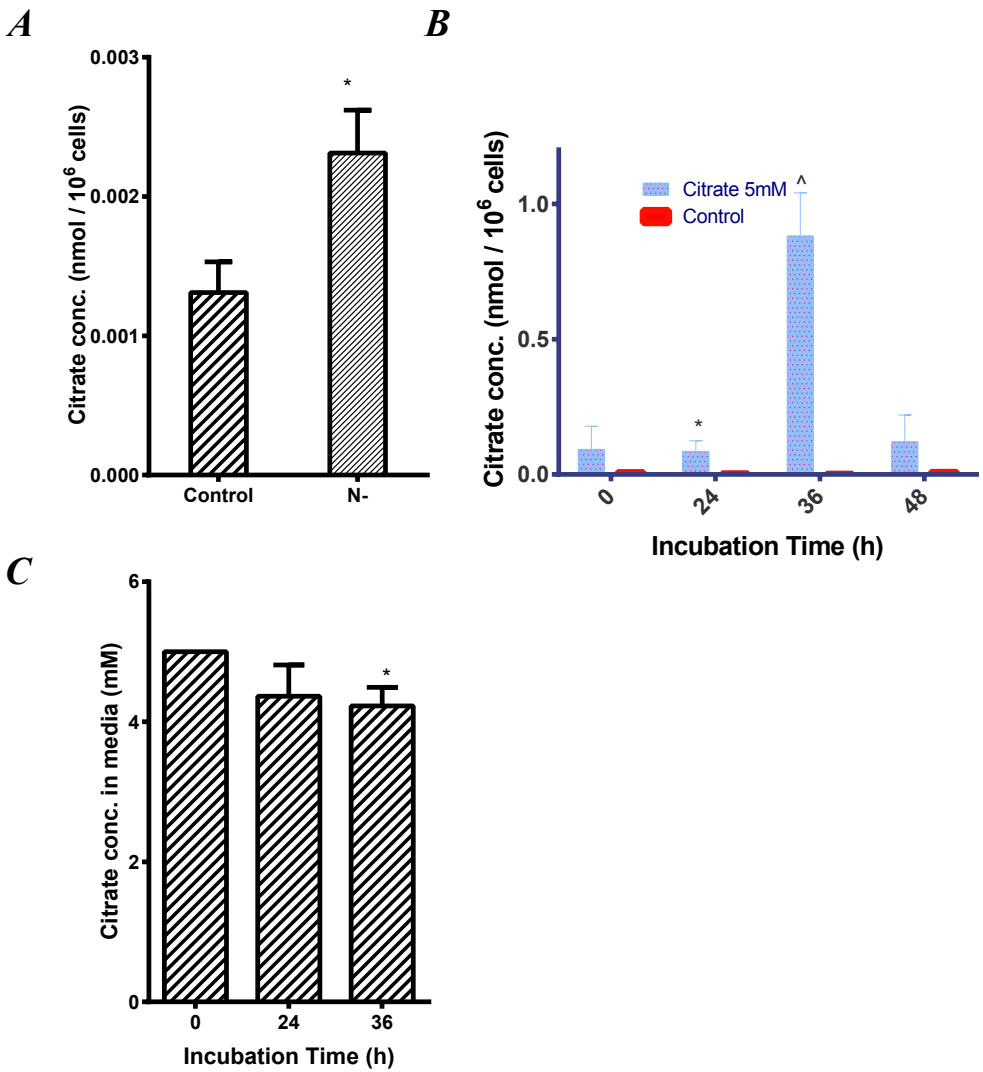


Figure 3.3: Change of citrate concentration over time. *: $p < 0.05$ and ^: $p < 0.01$. **A.** Effect of N starvation on the concentration of intracellular citrate. **B.** Change in citrate concentration in the cells. **C.** Change in citrate concentration in the media.

4 Transcriptome analysis using next-generation RNA sequencing

4 Transcriptome analysis using next-generation RNA sequencing

4.1 Quality assurance of RNA-seq experiment

4.2 Summary of overall differential gene expression patterns

4.3 Enrichment and pathway analysis

4.4 Validation of differential expression with qPCR

Quality assurance of RNA-seq experiment

- High-quality RNA was isolated with little degradation
- 88% of 168 million raw reads with satisfactory quality mapped to genome
- 19502 of 19526 annotated transcripts were assembled
- 5000 of putative novel isoforms were found

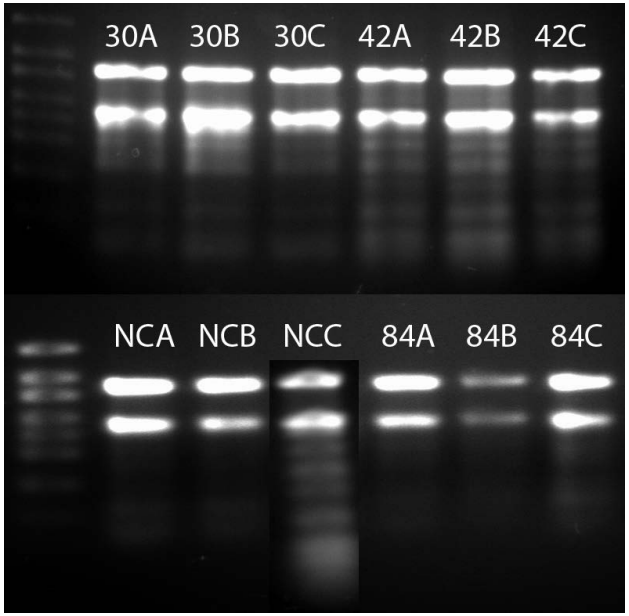
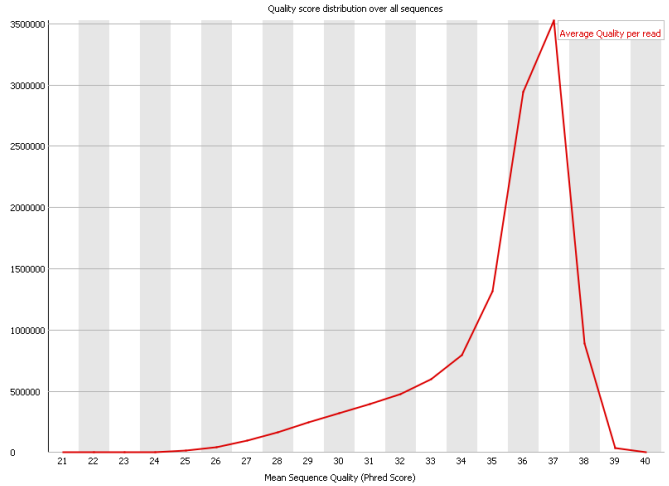


Figure 4.1: RNA gel electrophoresis image. Samples of RNA isolated from cells were check for integrity on a non-denaturing agarose gel. 28S and 18S rRNA were present in the top two bands, respectively, with an approximate density ratio of 2:1. Smear of degraded RNA was not observed.

A.



B.

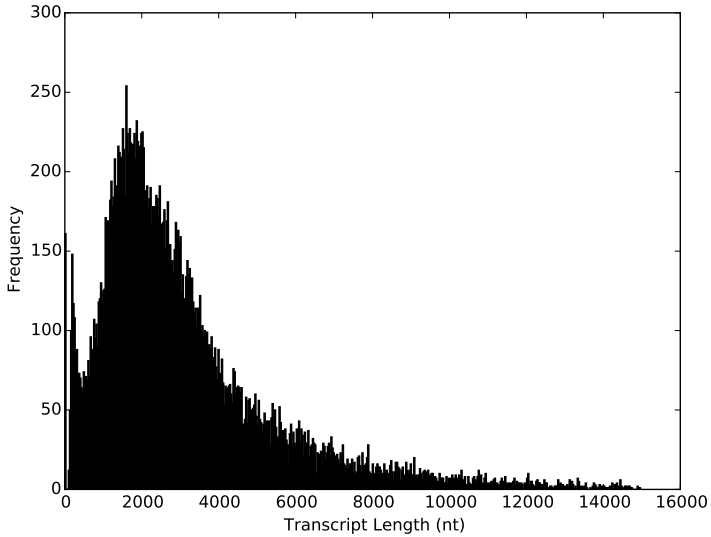


Figure 4.2: Quality check of raw sequencing reads and transcripts. **A.** Quality score distribution over all raw sequencing reads. **B.** Transcript length distribution over the assembly

4 Transcriptome analysis using next-generation RNA sequencing

Summary of overall differential gene expression patterns

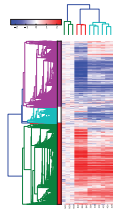
- Criteria: log2FC>2 or log2FC<-2, FDR < 0.01 and total CPM of all samples > 20
- Differentially expressed:
  - 6.24% of transcripts in cells treated with compound 30
  - 7.25% of transcripts in cells treated with compound 42
  - 16.12% of transcripts in cells treated with compound 84

Table 4.1: Numbers of differentially expressed transcripts

	Up	Down	Total	Percent change
30	754	462	1216	6.24%
42	917	496	1413	7.25%
84	1644	1500	3144	16.12%
30*[1]	36	53	89	0.46%
42*	51	29	80	0.41%
84*	806	1029	1835	9.41%
30+42*	69	62	131	0.67%
30+84*	36	66	102	0.52%
42+84*	183	125	308	1.58%
Common	613	280	893	4.58%

[1] * indicates that transcripts were differentially expressed only in the condition named in the current row. For example, the row of '42+84*' shows that 183 transcripts are significantly up-regulated in response to 42 and 84 but not 30.

A.



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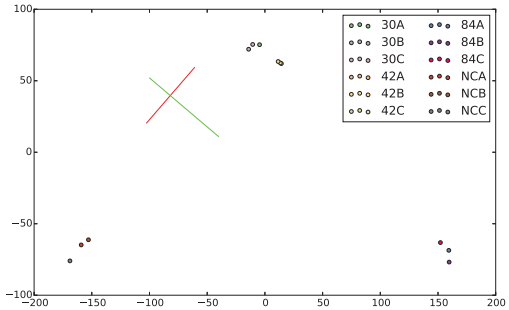
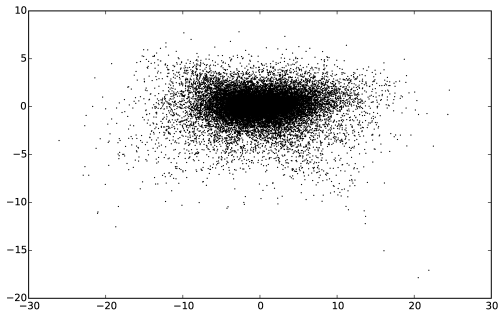


C.



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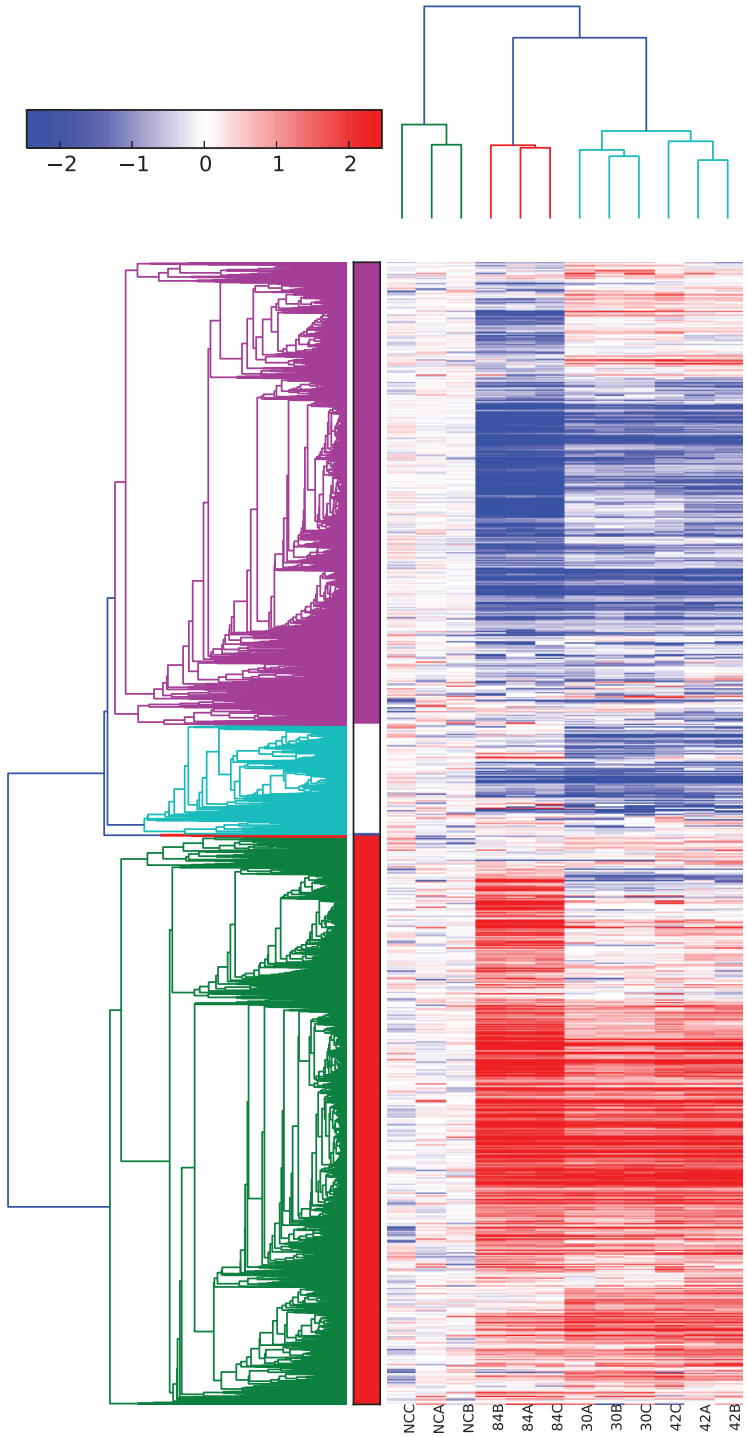
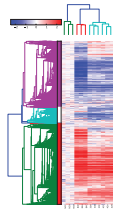
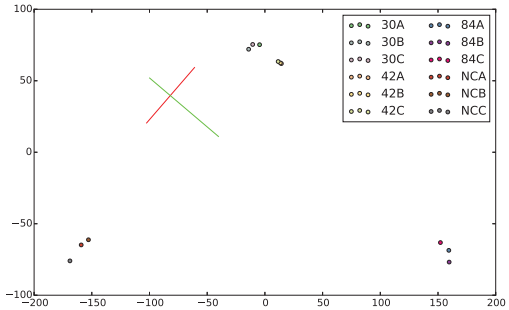


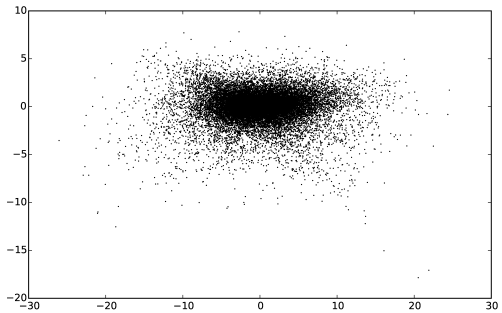
A.



B.



C.



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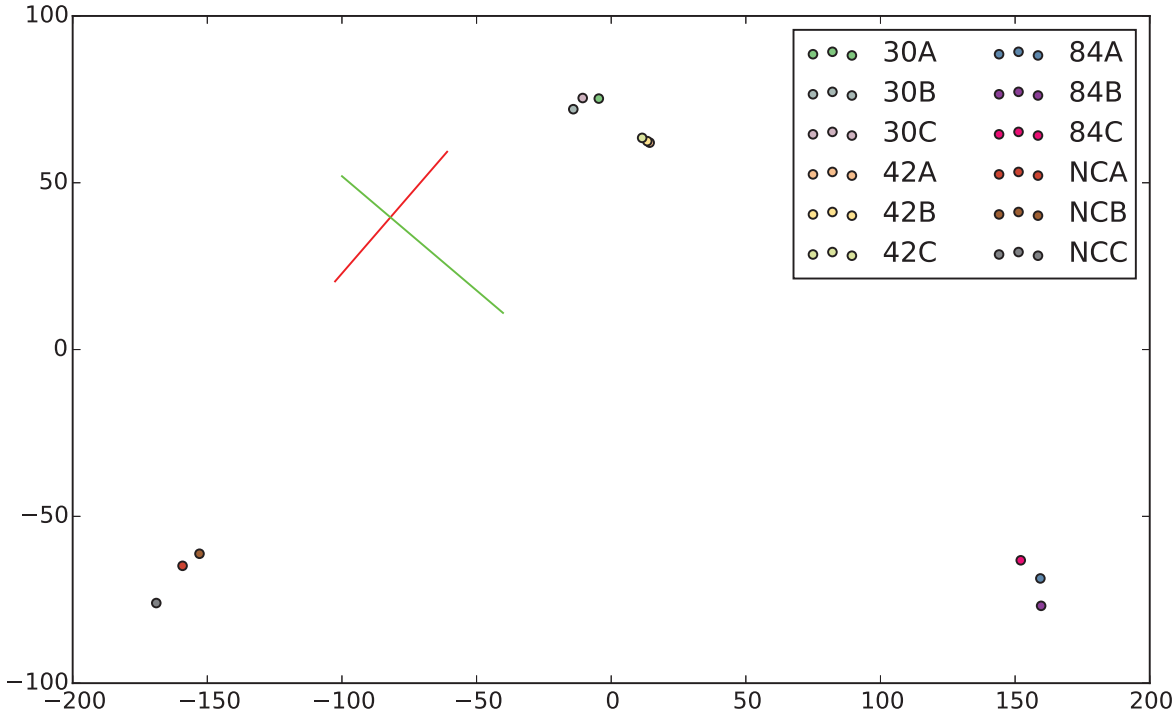
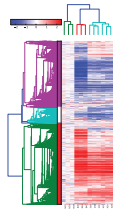

30A
30B
30C
42A
42B
42C
84A
84B
84C
NCA
NCB
NCC
100
50
0
−50
−100
−200
−150
−100
−50
0
50
100
150
200

A.



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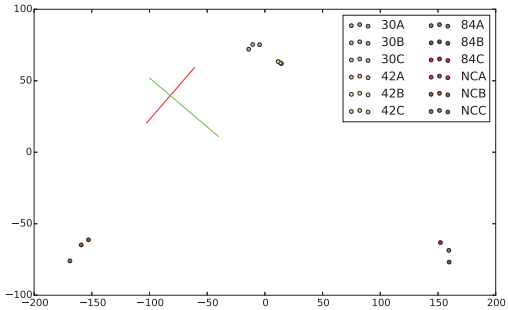

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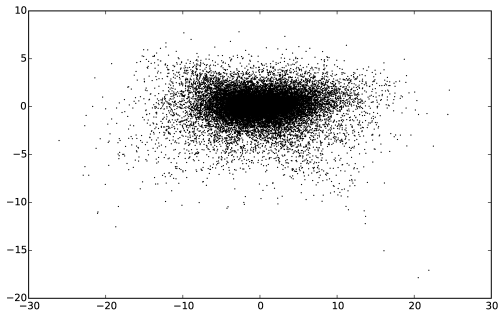

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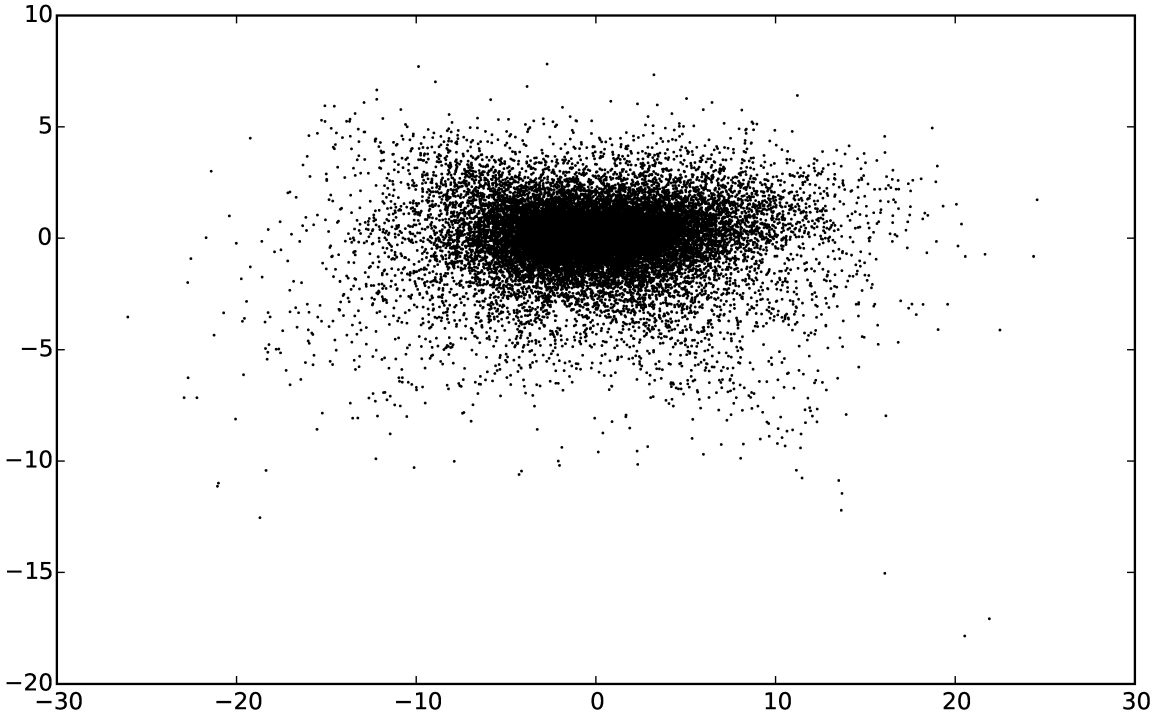

10
5
0
−5
−10
−15
−20
−30
−20
−10
0
10
20
30

4 Transcriptome analysis using next-generation RNA sequencing

Enrichment and pathway analysis

- Gene Set Enrichment Analysis based on GO terms:
  - GO term was assigned to each transcript based on protein domain information or homology
  - Gene sets were created based on these GO terms
- In-depth analysis of pathways related to lipid metabolism
  - Glycolysis
  - TCA cycle
  - Carbon fixation
  - Starch metabolism
  - Fatty acid biosynthesis
  - Lipid metabolism

Table 4.2: Summary of significantly changed GO categories

GO term	Size	NES(30)[1]	NES(42)	NES(84)
DNA replication	47	2.76*[2]	2.59*	2.59*
Vesicle-mediated transport	44	2.29*	2.26*	2.10*
Intracellular protein transport	66	2.26*	2.22*	1.98*
Microtubule-based movement	77	2.17*	1.62*	1.78*
DNA recombination	30	1.71*	1.68*	1.72*
ATP hydrolysis coupled proton transport	21	1.70*	1.61*	1.71*
(1→3)-β-D-glucan biosynthetic process	8	1.70*	1.67*	0.83
Glycerol metabolic process	14	1.57*	1.60*	1.94*
Proton transport	7	1.53*	1.2	1.4
tRNA aminoacylation for protein translation	39	1.51*	1.97*	2.26*
Protein ubiquitination	48	1.50*	1.61*	1.69*
Oxidation-reduction process	475	1.15	1.24	1.39*

[1] Normalized enrichment score for the condition specified. A positive score indicates up-regulation of the named gene set.

[2] * indicates nominal $p < 0.05$ and FDR < 0.25.

(continued)

GO term	Size	NES(30)	NES(42)	NES(84)
Photosynthesis light harvesting	30	-2.72*	-2.47*	-2.39*
Cyclic nucleotide biosynthetic process	181	-2.52*	-2.50*	-2.76*
Intracellular signal transduction	187	-2.41*	-2.36*	-2.66*
Translation	255	-1.91*	-2.27*	-1.54*
Phosphorelay signal transduction system	30	-1.90*	-2.17*	-2.37*
Photosynthesis	41	-1.83*	-1.57*	-2.14*
Riboflavin biosynthetic process	13	-1.74*	-1.51*	-1.45
Oxygen transport	13	-1.55*	-1.25	-1.29
Metal ion transport	39	-1.22	-1.07	-1.71*
Histidine biosynthetic process	9	-1.45	-1.63*	-0.49
Protein methylation	9	-1.46	-1.56	-1.71*

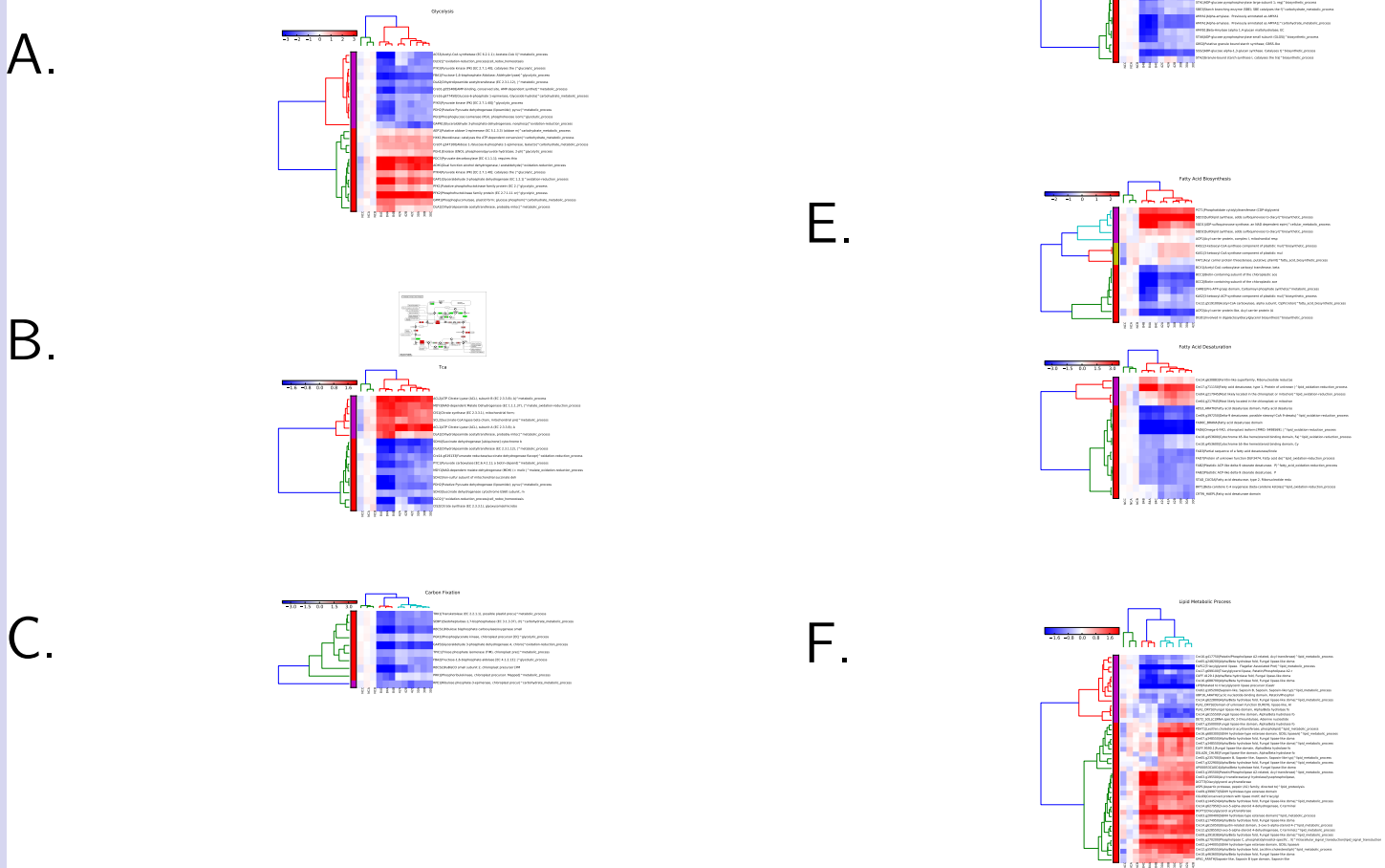
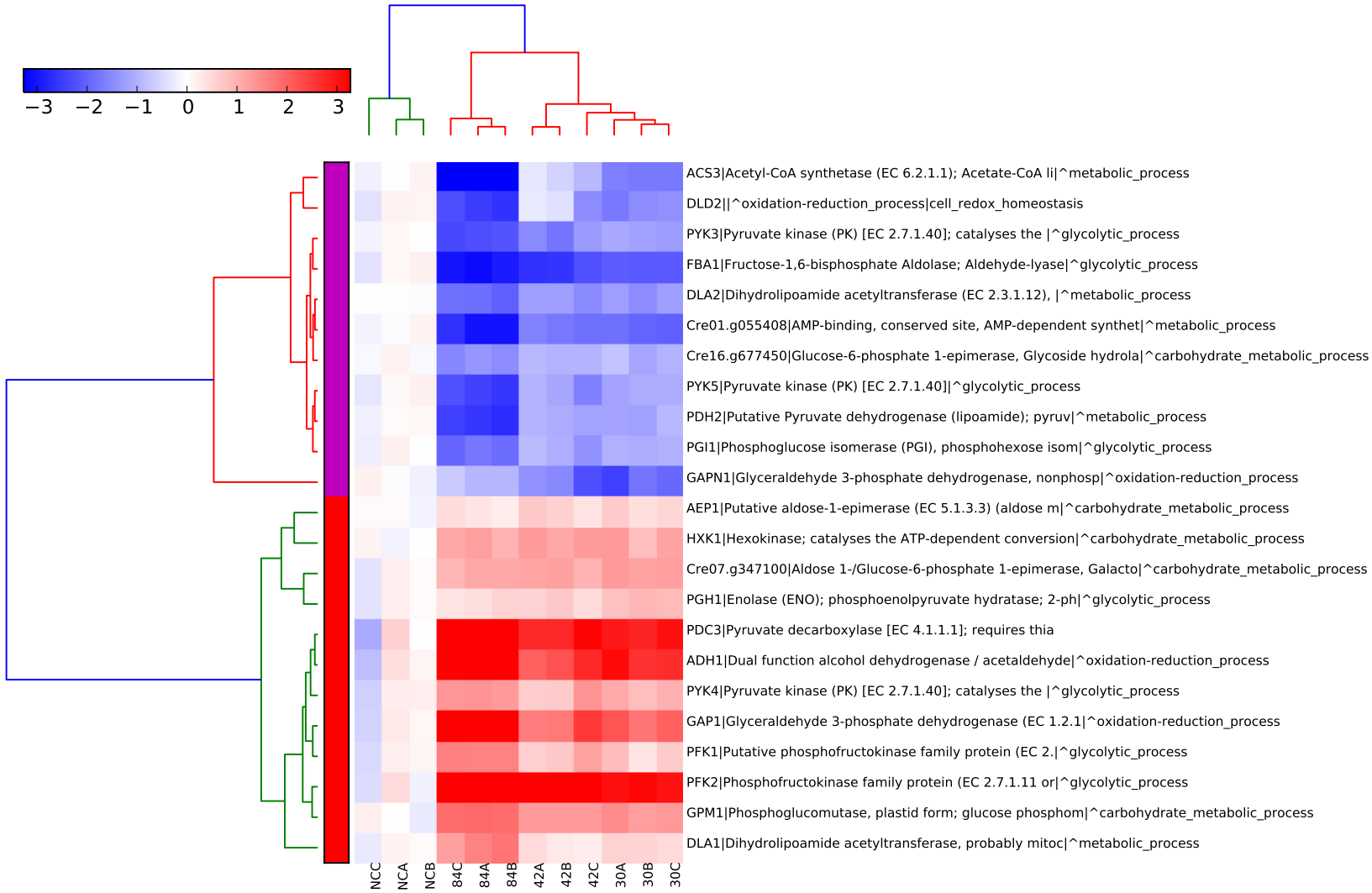
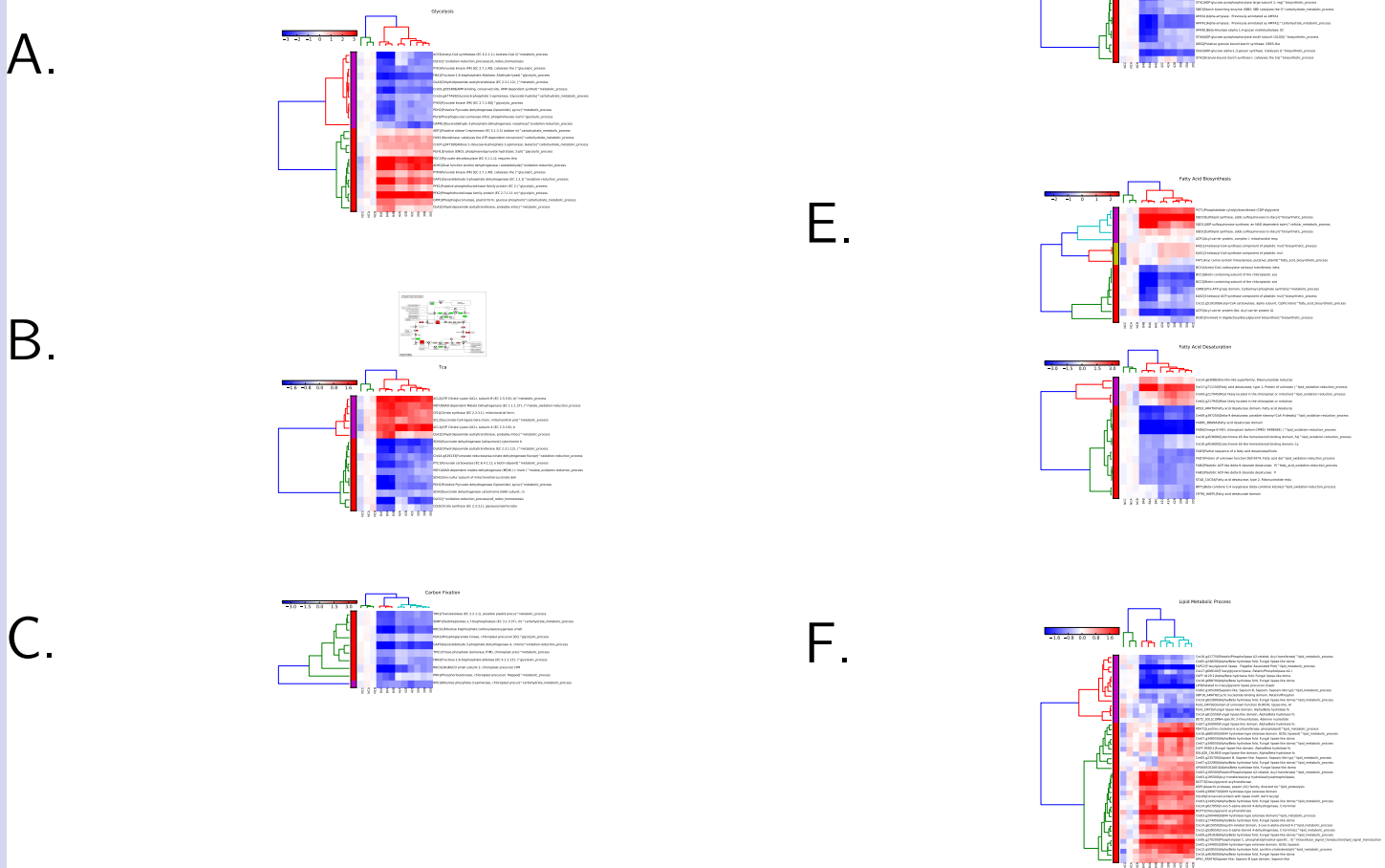


Figure 4.4: Expression profile of transcripts in coordinately shifted major metabolic pathways. Annotated transcripts encoding enzymes involved in specific pathways with total CPM > 10 and FDR < 0.01 in at least one condition were selected for analysis. **A.** Glycolysis. **B.** TCA cycle. **C.** Carbon fixation. **D.** Starch metabolism. **E.** Fatty acid biosynthesis **F.** Lipid metabolism.

Glycolysis


−3 −2 −1 0 1 2 3
ACS3|Acetyl-CoA synthetase (EC 6.2.1.1); Acetate-CoA li|^metabolic_process
DLD2||^oxidation-reduction_process|cell_redox_homeostasis
PYK3|Pyruvate kinase (PK) [EC 2.7.1.40]; catalyses the |^glycolytic_process
FBA1|Fructose-1,6-bisphosphate Aldolase; Aldehyde-lyase|^glycolytic_process
DLA2|Dihydrolipoamide acetyltransferase (EC 2.3.1.12), |^metabolic_process
Cre01.g055408|AMP-binding, conserved site, AMP-dependent synthet|^metabolic_process
Cre16.g677450|Glucose-6-phosphate 1-epimerase, Glycoside hydrola|^carbohydrate_metabolic_process
PYK5|Pyruvate kinase (PK) [EC 2.7.1.40]|^glycolytic_process
PDH2|Putative Pyruvate dehydrogenase (lipoamide); pyruv|^metabolic_process
PGI1|Phosphoglucose isomerase (PGI), phosphohexose isom|^glycolytic_process
GAPN1|Glyceraldehyde 3-phosphate dehydrogenase, nonphosp|^oxidation-reduction_process
AEP1|Putative aldose-1-epimerase (EC 5.1.3.3) (aldose m|^carbohydrate_metabolic_process
HXK1|Hexokinase; catalyses the ATP-dependent conversion|^carbohydrate_metabolic_process
Cre07.g347100|Aldose 1-/Glucose-6-phosphate 1-epimerase, Galacto|^carbohydrate_metabolic_process
PGH1|Enolase (ENO); phosphoenolpyruvate hydratase; 2-ph|^glycolytic_process
PDC3|Pyruvate decarboxylase [EC 4.1.1.1]; requires thia
ADH1|Dual function alcohol dehydrogenase / acetaldehyde|^oxidation-reduction_process
PYK4|Pyruvate kinase (PK) [EC 2.7.1.40]; catalyses the |^glycolytic_process
GAP1|Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1|^oxidation-reduction_process
PFK1|Putative phosphofructokinase family protein (EC 2.|^glycolytic_process
PFK2|Phosphofructokinase family protein (EC 2.7.1.11 or|^glycolytic_process
GPM1|Phosphoglucomutase, plastid form; glucose phosphom|^carbohydrate_metabolic_process
DLA1|Dihydrolipoamide acetyltransferase, probably mitoc|^metabolic_process
NCC
NCA
NCB
84C
84A
84B
42A
42B
42C
30A
30B
30C



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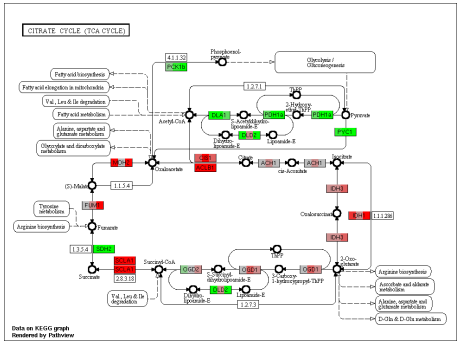
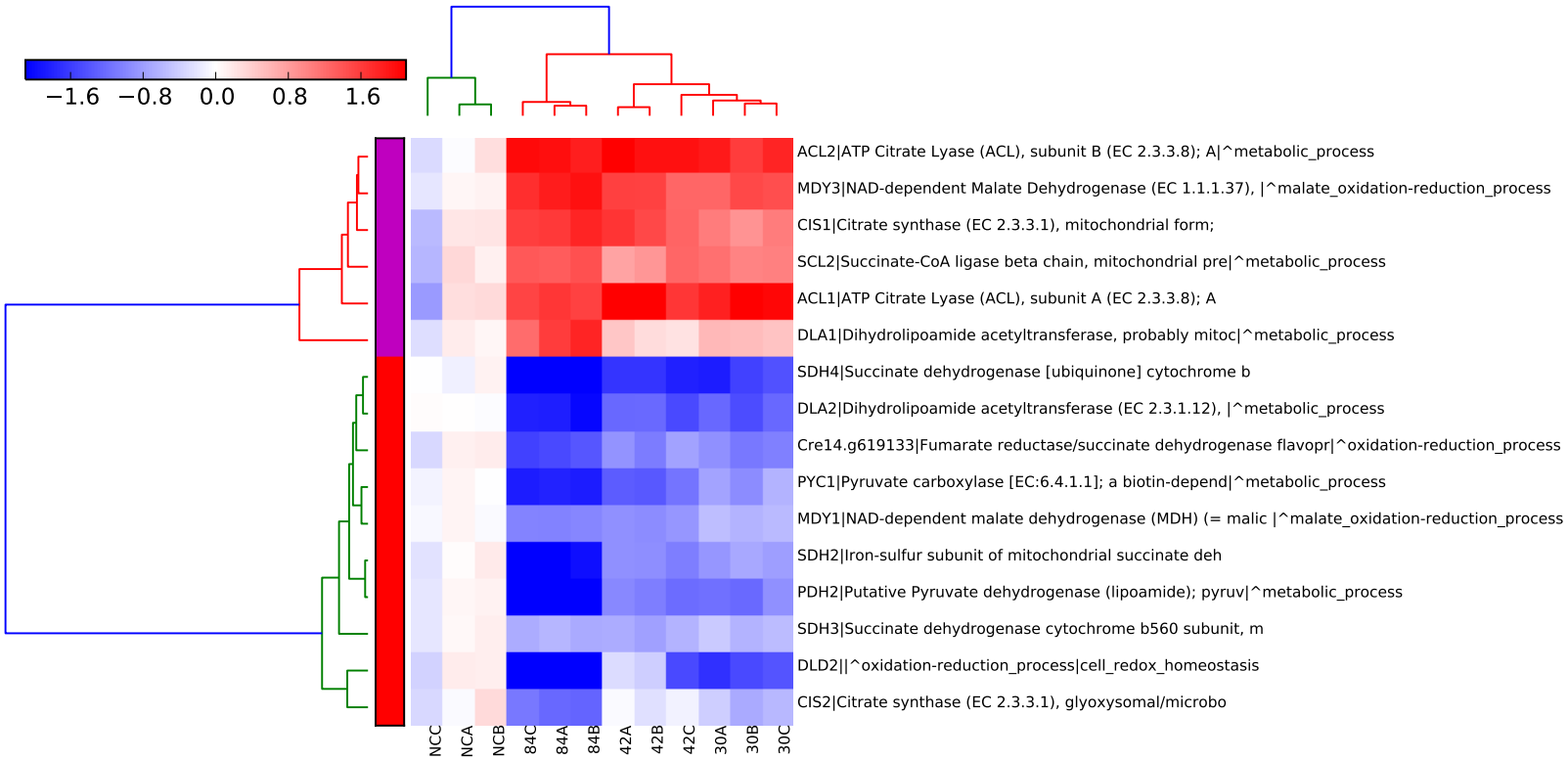
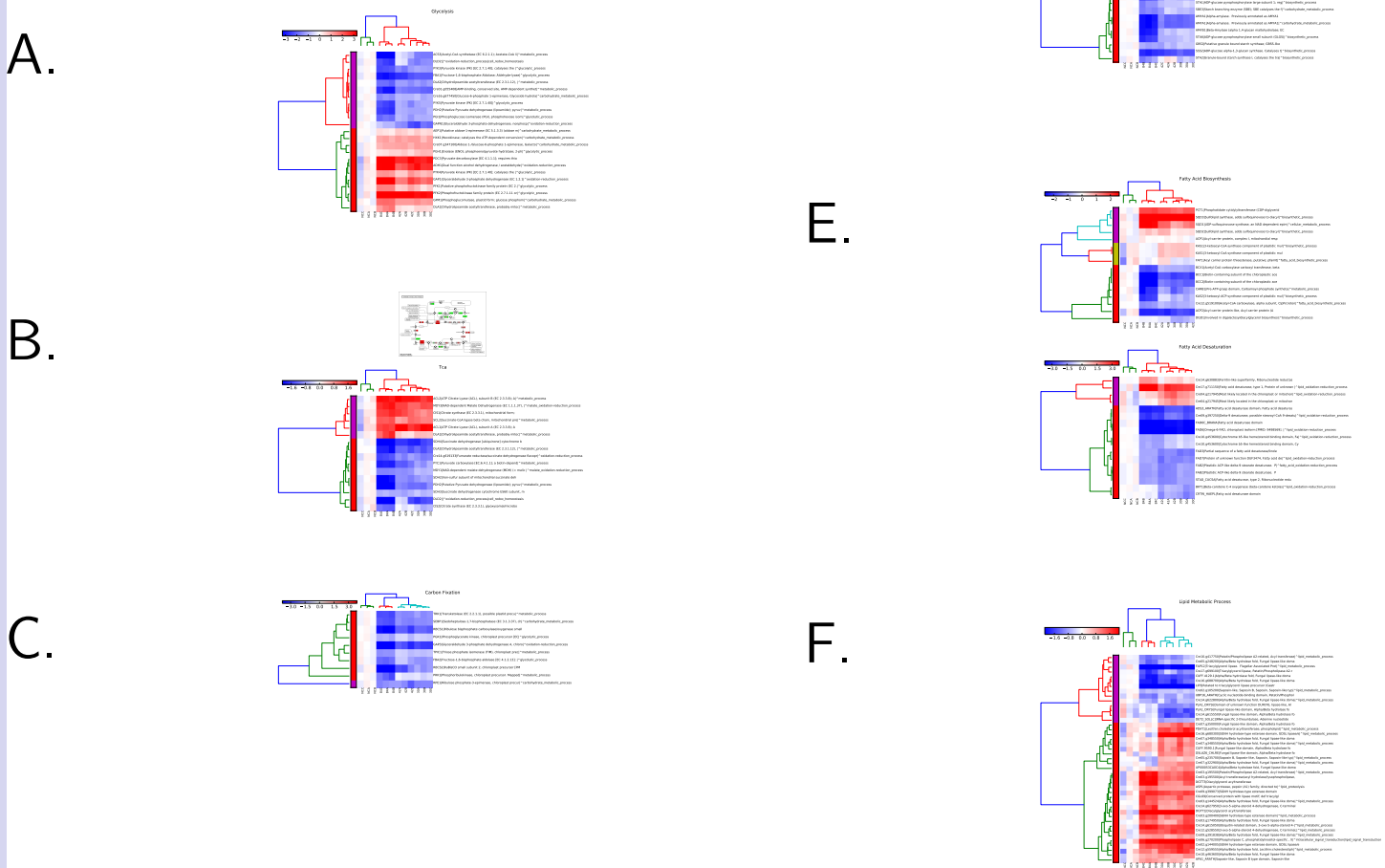

CITRATE CYCLE (TCA CYCLE)
Data on KEGG graph
Rendered by Pathview

Tca


−1.6 −0.8 0.0 0.8 1.6
ACL2|ATP Citrate Lyase (ACL), subunit B (EC 2.3.3.8); A|^metabolic_process
MDY3|NAD-dependent Malate Dehydrogenase (EC 1.1.1.37), |^malate_oxidation-reduction_process
CIS1|Citrate synthase (EC 2.3.3.1), mitochondrial form;
SCL2|Succinate-CoA ligase beta chain, mitochondrial pre|^metabolic_process
ACL1|ATP Citrate Lyase (ACL), subunit A (EC 2.3.3.8); A
DLA1|Dihydrolipoamide acetyltransferase, probably mitoc|^metabolic_process
SDH4|Succinate dehydrogenase [ubiquinone] cytochrome b
DLA2|Dihydrolipoamide acetyltransferase (EC 2.3.1.12), |^metabolic_process
Cre14.g619133|Fumarate reductase/succinate dehydrogenase flavopr|^oxidation-reduction_process
PYC1|Pyruvate carboxylase [EC:6.4.1.1]; a biotin-depend|^metabolic_process
MDY1|NAD-dependent malate dehydrogenase (MDH) (= malic |^malate_oxidation-reduction_process
SDH2|Iron-sulfur subunit of mitochondrial succinate deh
PDH2|Putative Pyruvate dehydrogenase (lipoamide); pyruv|^metabolic_process
SDH3|Succinate dehydrogenase cytochrome b560 subunit, m
DLD2||^oxidation-reduction_process|cell_redox_homeostasis
CIS2|Citrate synthase (EC 2.3.3.1), glyoxysomal/microbo
NCC
NCA
NCB
84C
84A
84B
42A
42B
42C
30A
30B
30C



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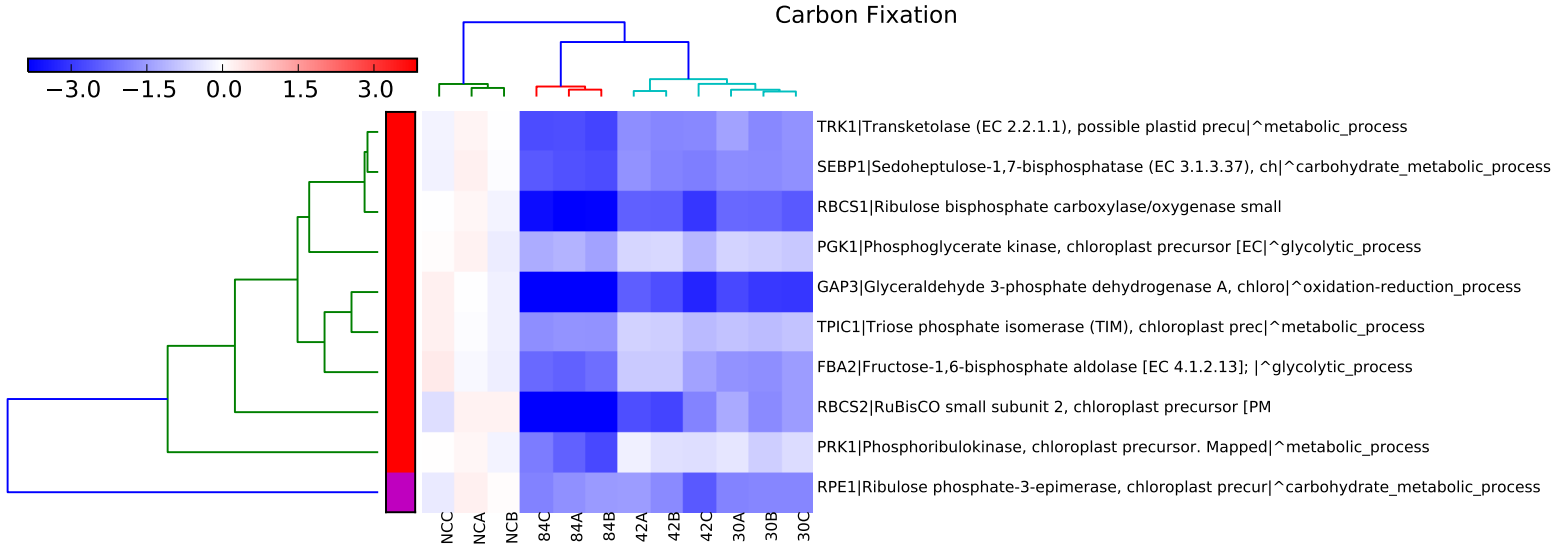
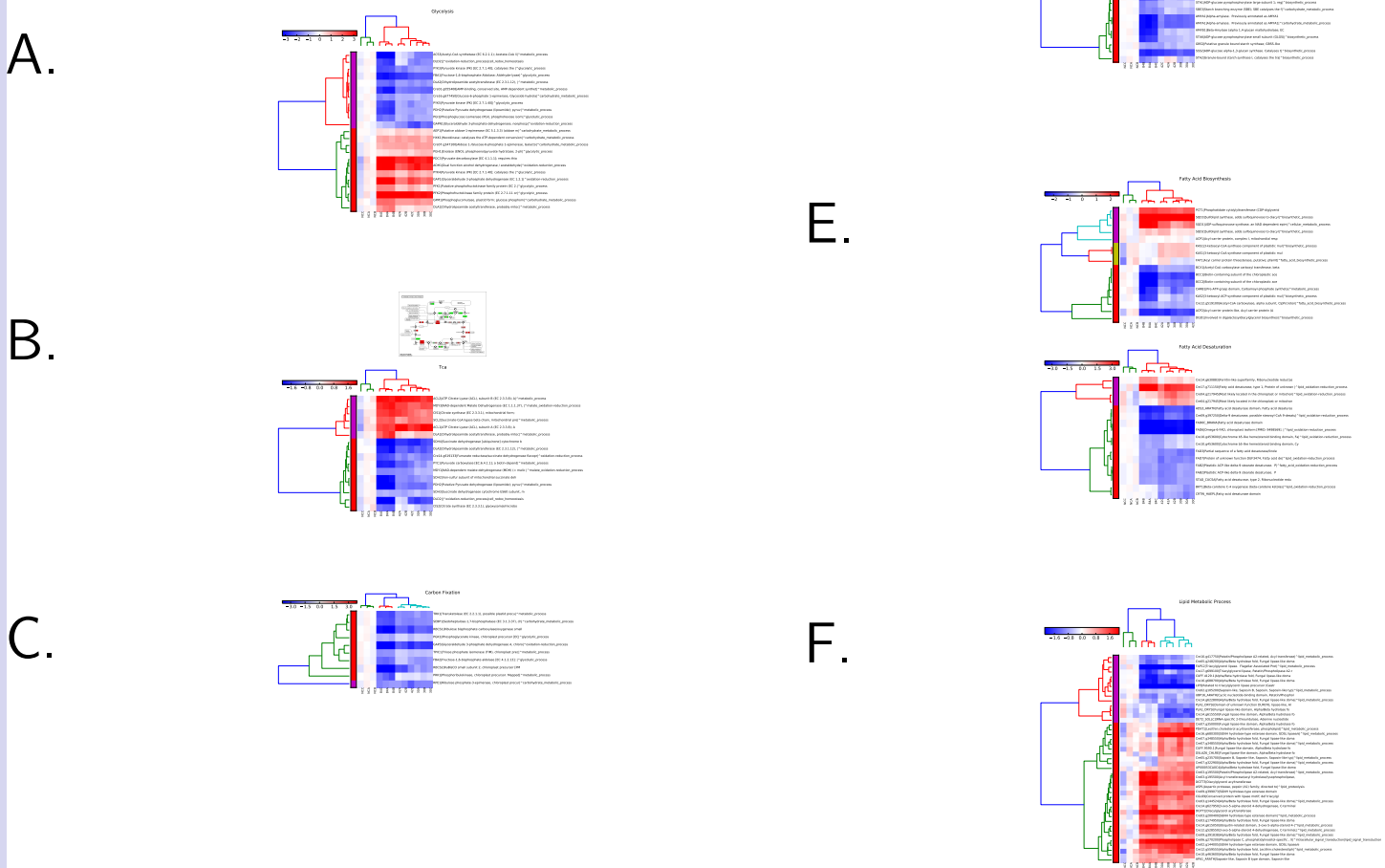

Carbon Fixation
−3.0
−1.5
0.0
1.5
3.0
TRK1|Transketolase (EC 2.2.1.1), possible plastid precu|^metabolic_process
SEBP1|Sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37), ch|^carbohydrate_metabolic_process
RBCS1|Ribulose bisphosphate carboxylase/oxygenase small
PGK1|Phosphoglycerate kinase, chloroplast precursor [EC|^glycolytic_process
GAP3|Glyceraldehyde 3-phosphate dehydrogenase A, chloro|^oxidation-reduction_process
TPIC1|Triose phosphate isomerase (TIM), chloroplast prec|^metabolic_process
FBA2|Fructose-1,6-bisphosphate aldolase [EC 4.1.2.13]; |^glycolytic_process
RBCS2|RuBisCO small subunit 2, chloroplast precursor [PM
PRK1|Phosphoribulokinase, chloroplast precursor. Mapped|^metabolic_process
RPE1|Ribulose phosphate-3-epimerase, chloroplast precur|^carbohydrate_metabolic_process
NCC
NCA
NCB
84C
84A
84B
42A
42B
42C
30A
30B
30C



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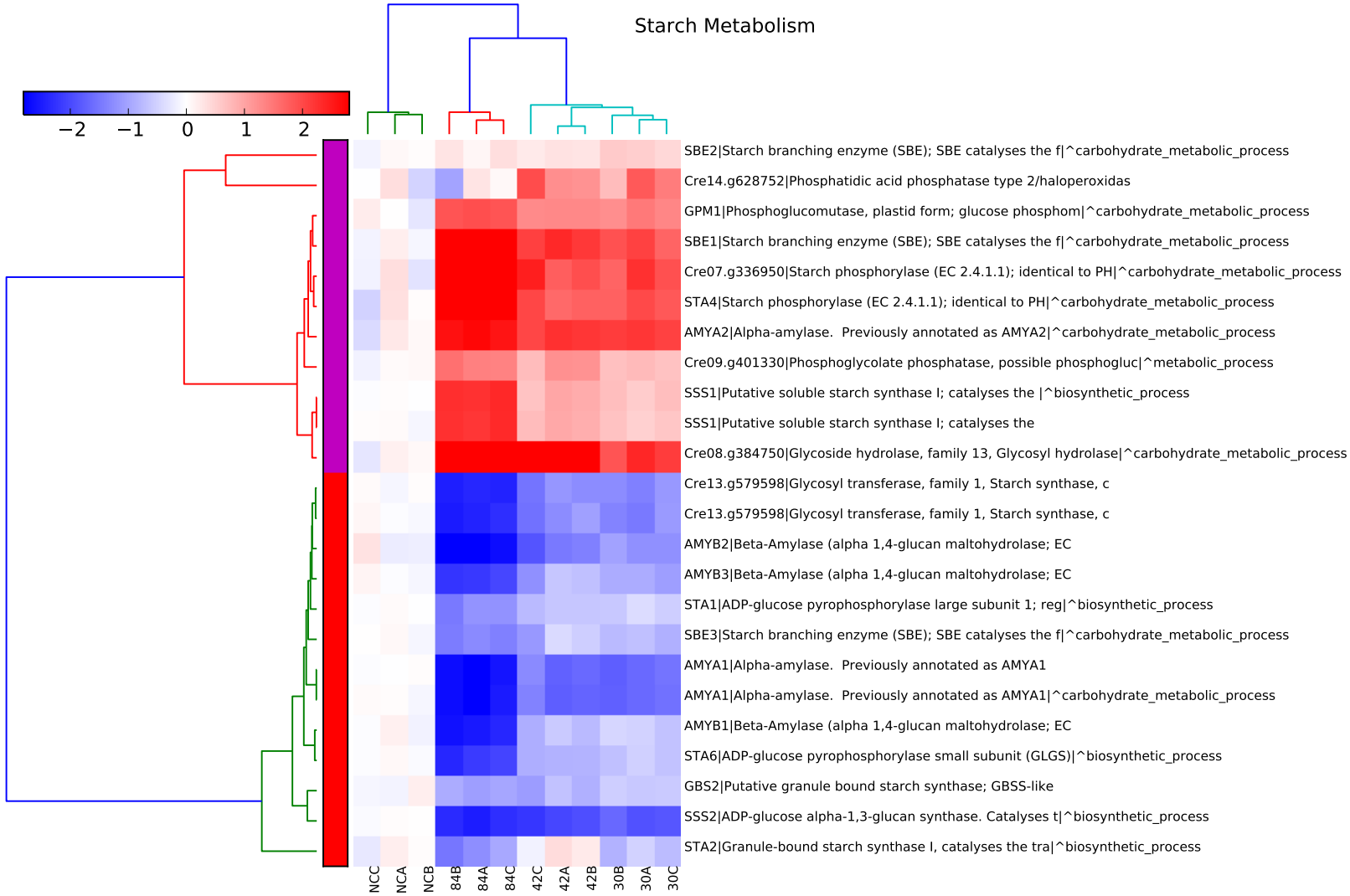
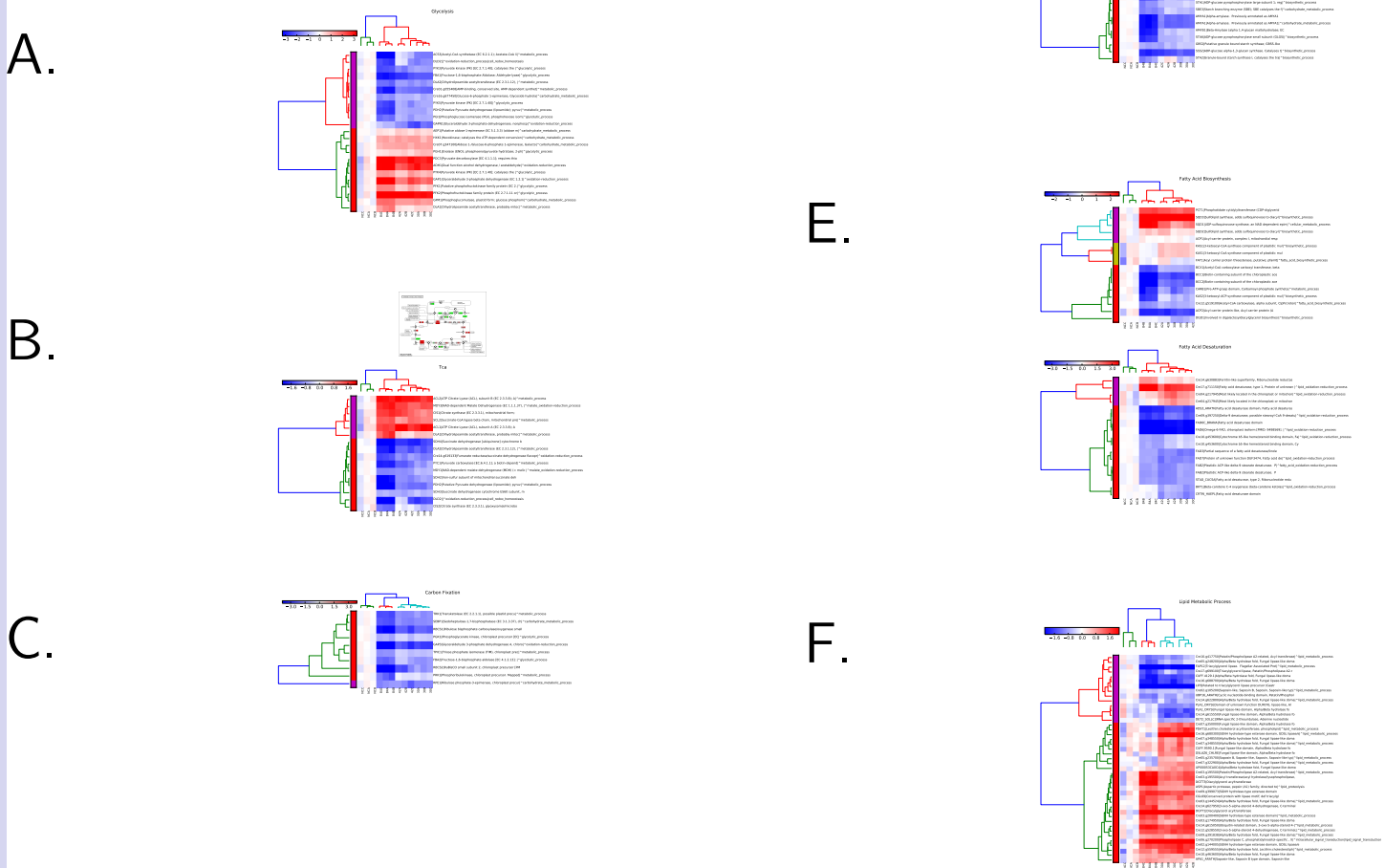

Starch Metabolism
−2
−1
0
1
2
SBE2|Starch branching enzyme (SBE); SBE catalyses the f|^carbohydrate_metabolic_process
Cre14.g628752|Phosphatidic acid phosphatase type 2/haloperoxidas
GPM1|Phosphoglucomutase, plastid form; glucose phosphom|^carbohydrate_metabolic_process
SBE1|Starch branching enzyme (SBE); SBE catalyses the f|^carbohydrate_metabolic_process
Cre07.g336950|Starch phosphorylase (EC 2.4.1.1); identical to PH|^carbohydrate_metabolic_process
STA4|Starch phosphorylase (EC 2.4.1.1); identical to PH|^carbohydrate_metabolic_process
AMYA2|Alpha-amylase. Previously annotated as AMYA2|^carbohydrate_metabolic_process
Cre09.g401330|Phosphoglycolate phosphatase, possible phosphogluc|^metabolic_process
SSS1|Putative soluble starch synthase I; catalyses the |^biosynthetic_process
SSS1|Putative soluble starch synthase I; catalyses the
Cre08.g384750|Glycoside hydrolase, family 13, Glycosyl hydrolase|^carbohydrate_metabolic_process
Cre13.g579598|Glycosyl transferase, family 1, Starch synthase, c
Cre13.g579598|Glycosyl transferase, family 1, Starch synthase, c
AMYB2|Beta-Amylase (alpha 1,4-glucan maltohydrolase; EC
AMYB3|Beta-Amylase (alpha 1,4-glucan maltohydrolase; EC
STA1|ADP-glucose pyrophosphorylase large subunit 1; reg|^biosynthetic_process
SBE3|Starch branching enzyme (SBE); SBE catalyses the f|^carbohydrate_metabolic_process
AMYA1|Alpha-amylase. Previously annotated as AMYA1
AMYA1|Alpha-amylase. Previously annotated as AMYA1|^carbohydrate_metabolic_process
AMYB1|Beta-Amylase (alpha 1,4-glucan maltohydrolase; EC
STA6|ADP-glucose pyrophosphorylase small subunit (GLGS)|^biosynthetic_process
GBS2|Putative granule bound starch synthase; GBSS-like
SSS2|ADP-glucose alpha-1,3-glucan synthase. Catalyses t|^biosynthetic_process
STA2|Granule-bound starch synthase I, catalyses the tra|^biosynthetic_process
NCC
NCA
NCB
84B
84A
84C
42C
42A
42B
30B
30A
30C



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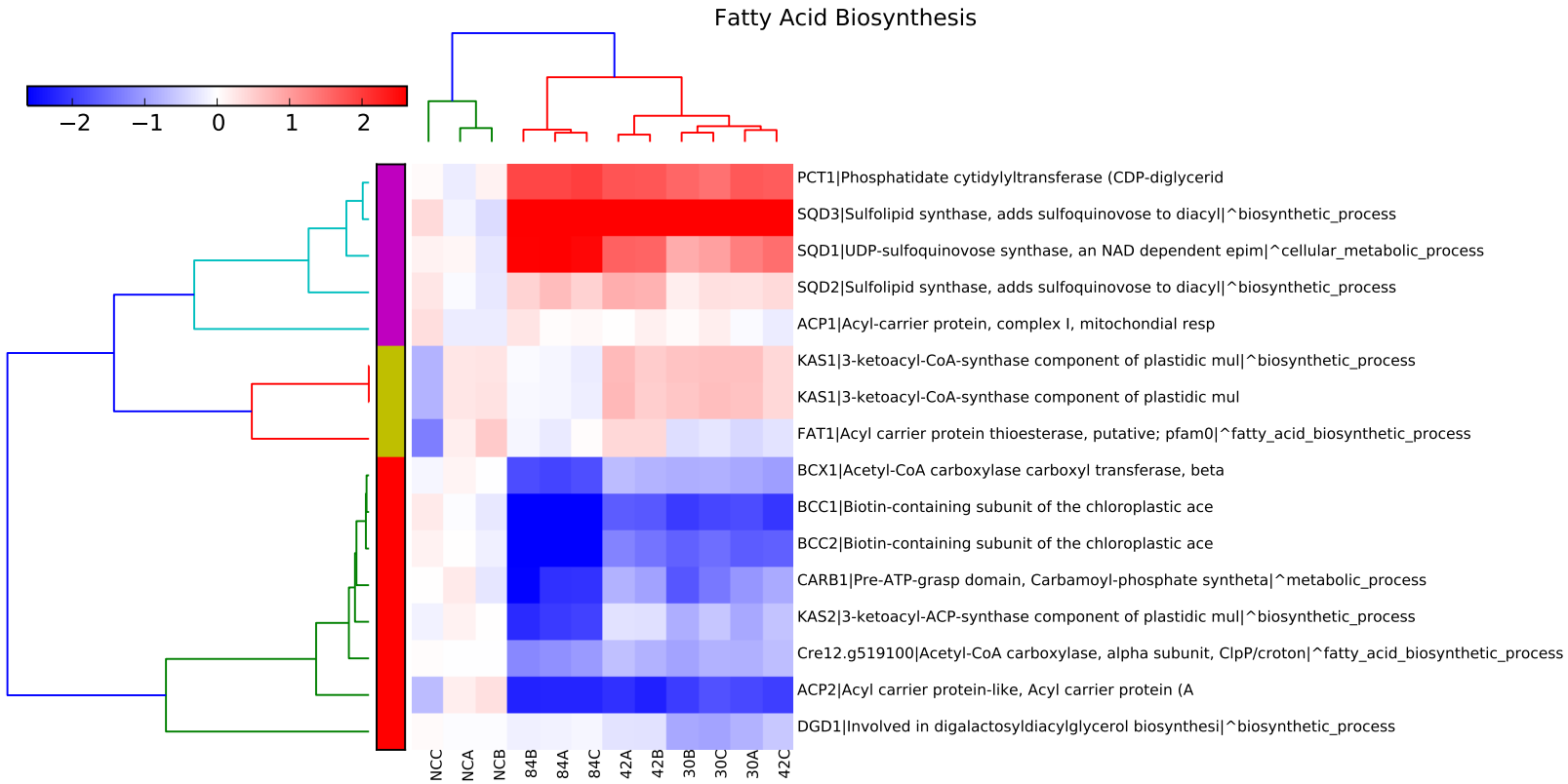
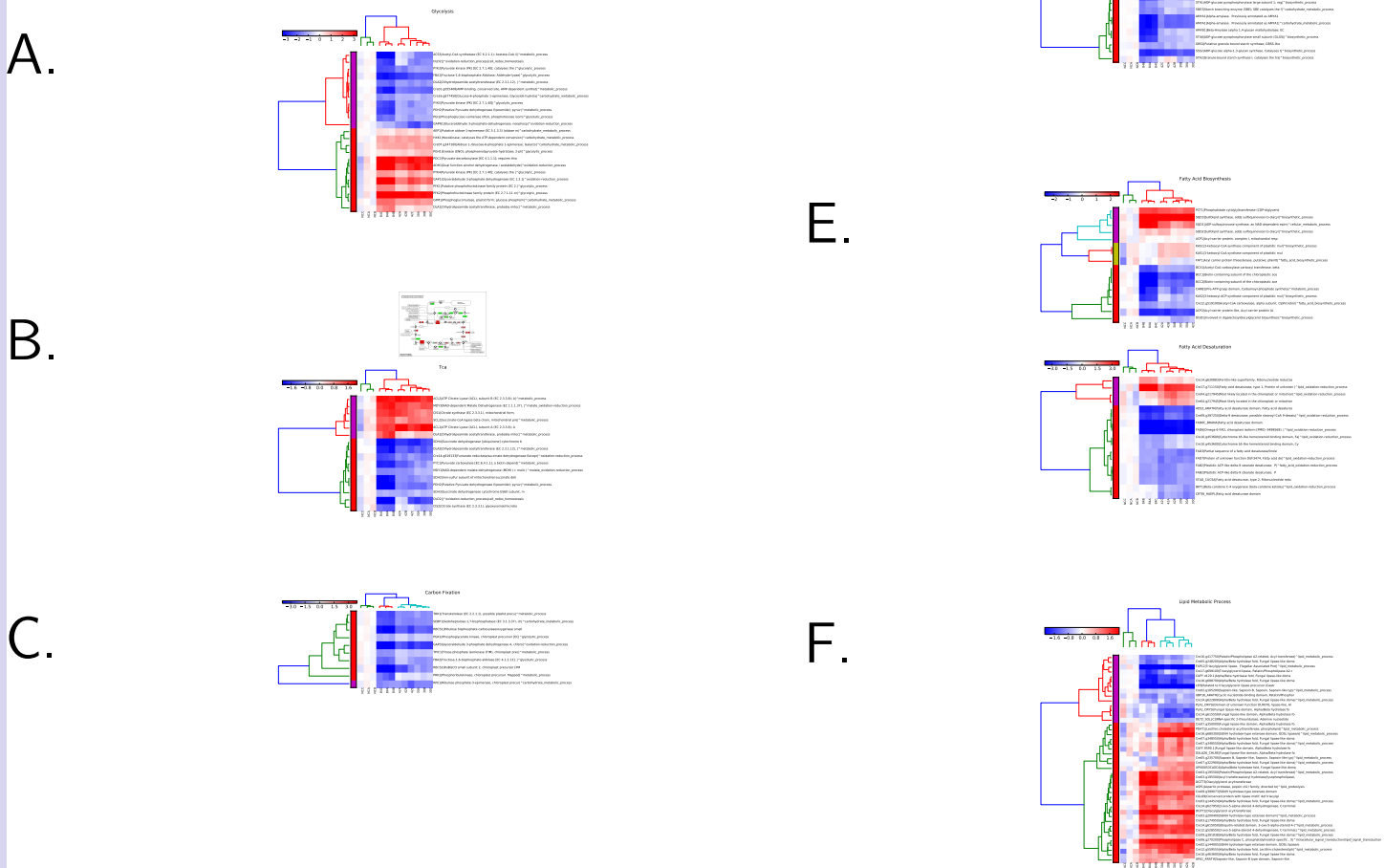

Fatty Acid Biosynthesis
−2
−1
0
1
2
PCT1|Phosphatidate cytidylyltransferase (CDP-diglycerid
SQD3|Sulfolipid synthase, adds sulfoquinovose to diacyl|^biosynthetic_process
SQD1|UDP-sulfoquinovose synthase, an NAD dependent epim|^cellular_metabolic_process
SQD2|Sulfolipid synthase, adds sulfoquinovose to diacyl|^biosynthetic_process
ACP1|Acyl-carrier protein, complex I, mitochondial resp
KAS1|3-ketoacyl-CoA-synthase component of plastidic mul|^biosynthetic_process
KAS1|3-ketoacyl-CoA-synthase component of plastidic mul
FAT1|Acyl carrier protein thioesterase, putative; pfam0|^fatty_acid_biosynthetic_process
BCX1|Acetyl-CoA carboxylase carboxyl transferase, beta
BCC1|Biotin-containing subunit of the chloroplastic ace
BCC2|Biotin-containing subunit of the chloroplastic ace
CARB1|Pre-ATP-grasp domain, Carbamoyl-phosphate syntheta|^metabolic_process
KAS2|3-ketoacyl-ACP-synthase component of plastidic mul|^biosynthetic_process
Cre12.g519100|Acetyl-CoA carboxylase, alpha subunit, ClpP/croton|^fatty_acid_biosynthetic_process
ACP2|Acyl carrier protein-like, Acyl carrier protein (A
DGD1|Involved in digalactosyldiacylglycerol biosynthesi|^biosynthetic_process
NCC
NCA
NCB
84B
84A
84C
42A
42B
30B
30C
30A
42C



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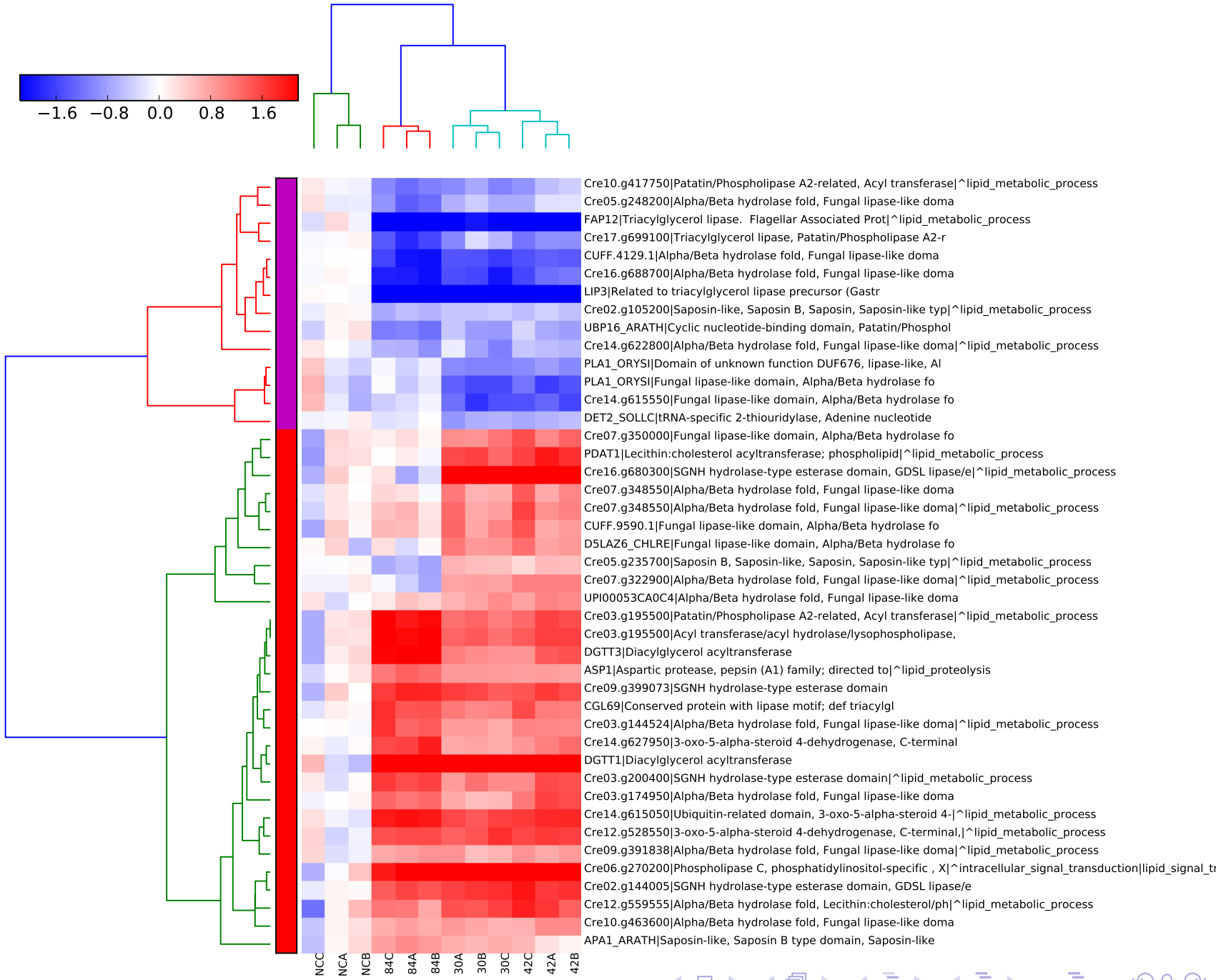
Lipid Metabolic Process



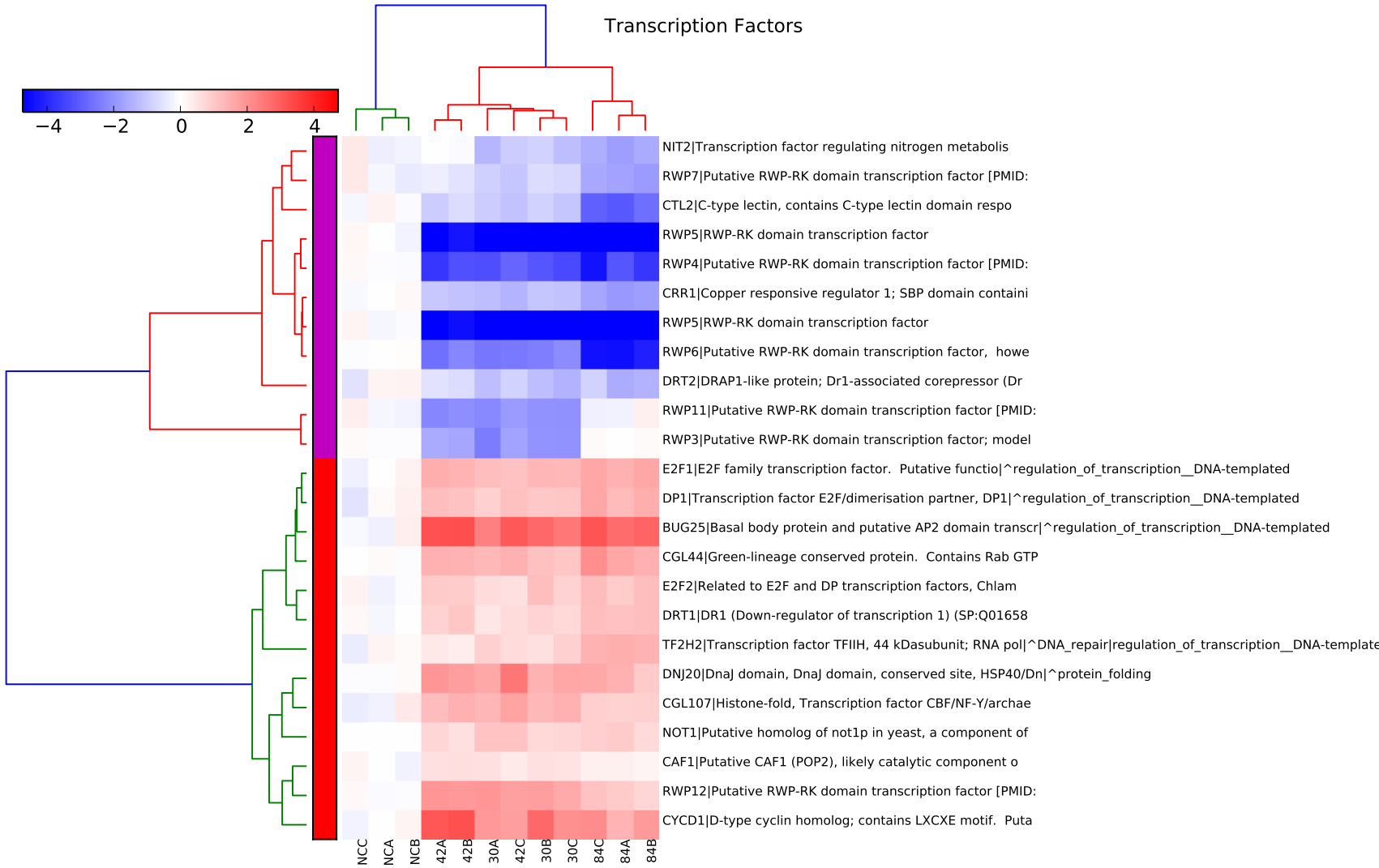


Figure 4.5: Expression profile of transcription factors. Annotated transcripts encoding transcription factors with total CPM > 10 and FDR < 0.01 in at least one condition were selected for analysis.

4 Transcriptome analysis using next-generation RNA sequencing

4.1 Quality assurance of RNA-seq experiment

4.2 Summary of overall differential gene expression patterns

4.3 Enrichment and pathway analysis

4.4 Validation of differential expression with qPCR

Validation of differential expression with qPCR

- 4 genes of interest expressed at different levels were selected
- Results from RNA-seq are comparable to qPCR data

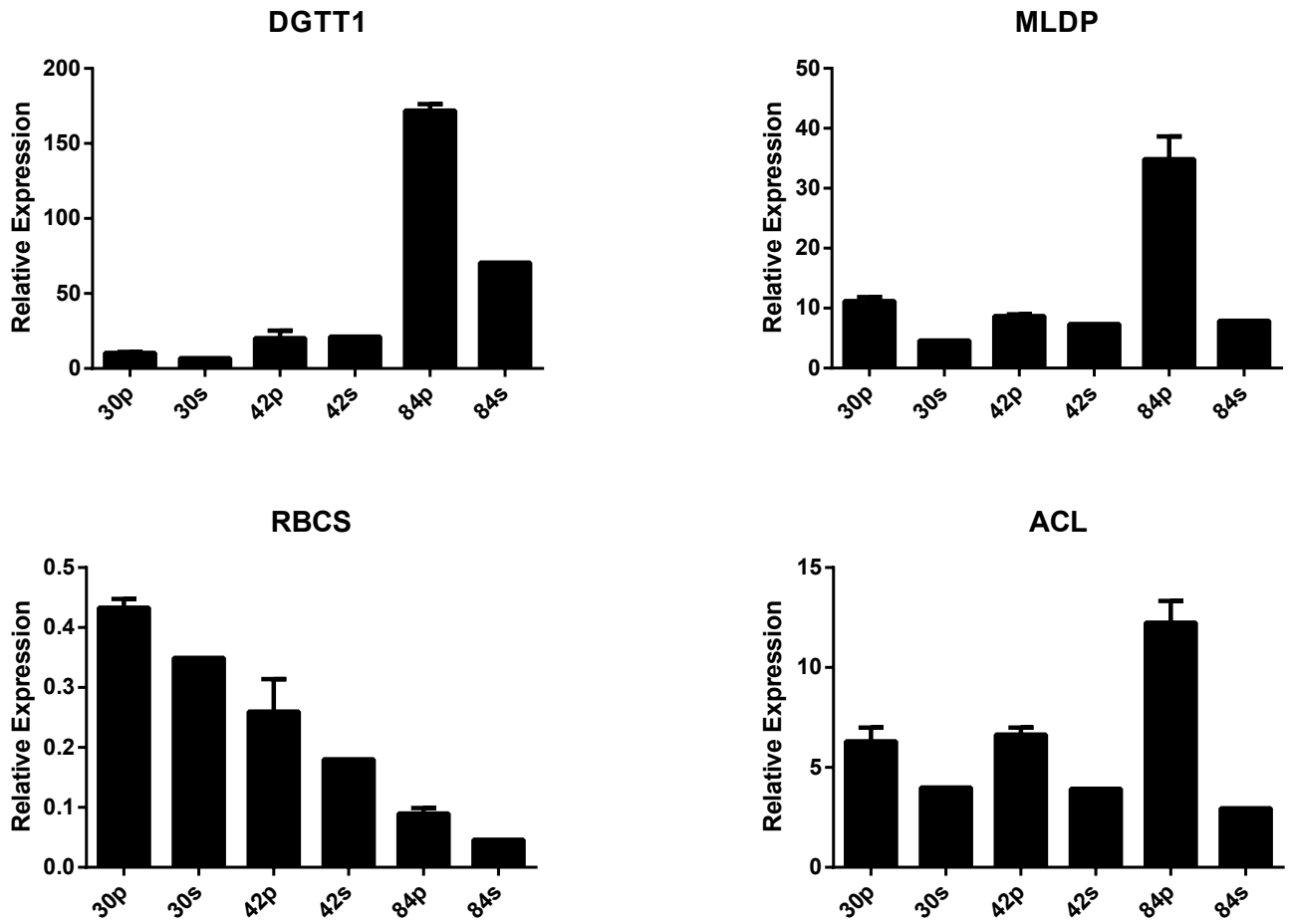


Figure 4.6: Comparison of the expression of selected genes using qPCR. In qPCR measurements (indicated with suffix "p"), relative expression is the fold change of each condition relative to control (N+) normalized with the relative expression of reference gene RACK1. In RNA-seq measurements (indicated with suffix "s"), relative expression is the fold change of CPM in each condition relative to control.

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5 Discussion

5 Discussion

Effects of compounds on lipid accumulation and growth

- Four selected compounds induce significant increase in TAG accumulation in *Chlamydomonas reinhardtii* without severe impact on growth
- Compound 84 induced a 6-fold increase in lipid droplet accumulation
- Compound 30, 42 and 67 induced 3- to 5-fold increase
- Except for compound 84, growth reduction was only between 11% to 18%
- Cells were treated with 20 µM of each compound. EC50: 5 µM

5 Discussion

Advantages and limitations of RNA-seq and computational methods

- Illumina HiSeq: affordable cost and high reliability. 30 million reads for each sample: 100 bp single-end
- Computational tools were carefully selected based on the efficiency and suitability of the algorithms
- 19502 out of 19526 annotated transcripts were detected
- 5335 putative novel transcripts were also assembled
- Alternative splicing analysis was not performed as pair-end reads were not used

5 Discussion

Overview of the transcriptional responses leading to TAG accumulation

- Metabolic fluxes are channeled to TAG accumulation in a mechanism distinct from nitrogen starvation
- Carbon flow from glycolysis: fermentation $>$ TCA cycle
- ACS3 $\downarrow$, encoding acetyl-CoA synthase. Genes in starch catabolism $\uparrow$
- Citrate efflux from TCA cycle contributes to fatty acid biosynthesis
- Turnover of SQDG and PG but not DGDG may play important role in TAG accumulation
- Strong suppression of genes encoding TAG lipases

5 Discussion

Cell signaling and transcription regulation

- RWP-RK domain TFs, regulators of nitrogen responses and gametogenesis $\downarrow$: e.g. NIT2
- SBP domain TFs, regulators of fatty acid and lipid biosynthesis $\downarrow$: e.g. NRR1
- TFs regulating cell cycle progression $\uparrow$
- NIT2 $\downarrow \rightarrow$ NIA1 $\downarrow \rightarrow$ NO_3^- $\uparrow$
- SAC3 $\uparrow \rightarrow$ ASR1/3 $\uparrow \rightarrow$ SO_4^- $\uparrow$

5 Discussion

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Future directions

- Biological processes on protein and metabolite level: targeted proteomics and metabolics
- Lipid trafficking and biosynthetic process: lipidomics
- Cell signaling cascade: protein phosphorylation and small effectors
- Direct target identification: protein-ligand interaction