

Supplementary information:

Title

Understanding the regulation of overwintering diapause molecular mechanisms in *Culex pipiens pallens* through comparative proteomics

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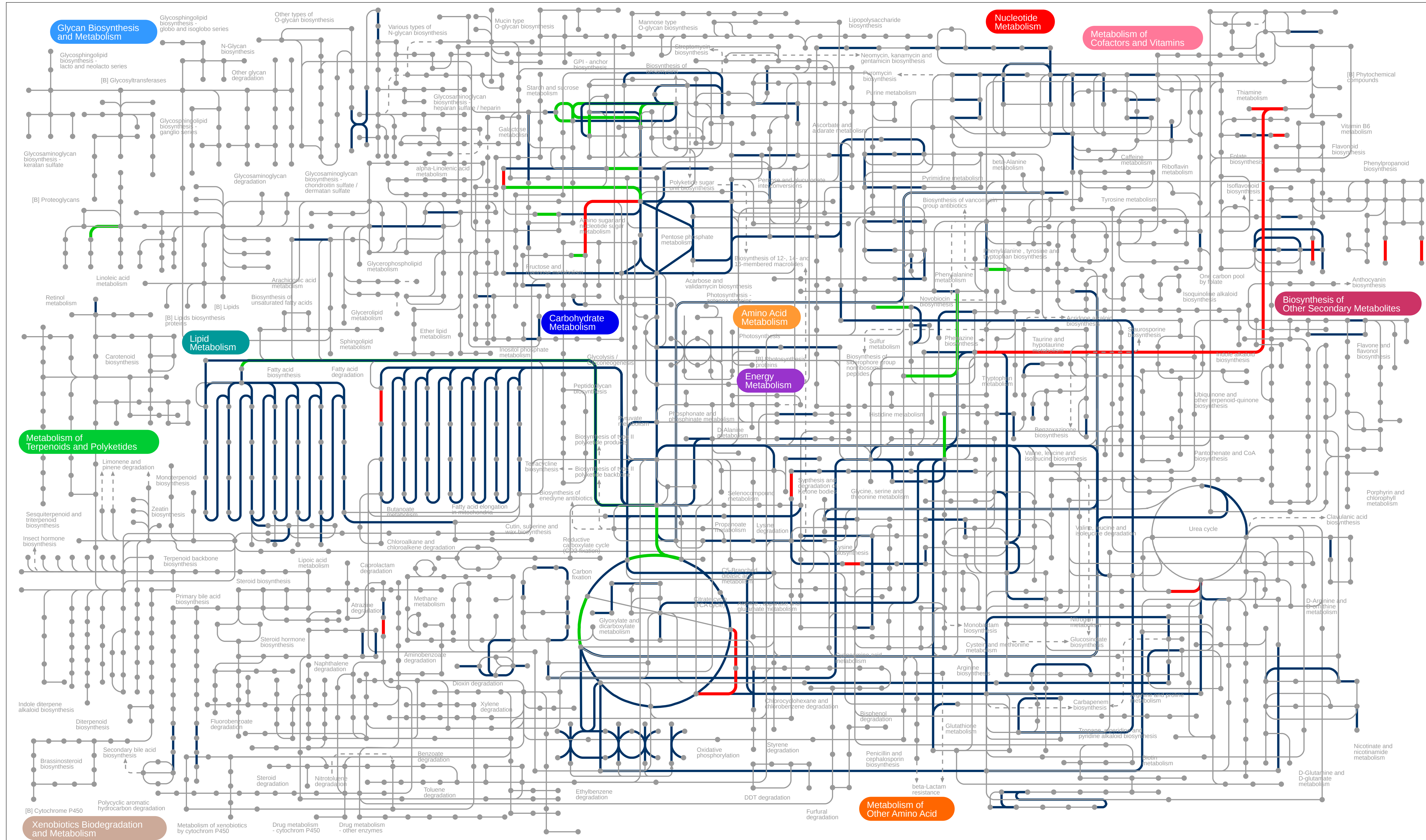
3 Shanghai MHelix BioTech Co., Ltd.

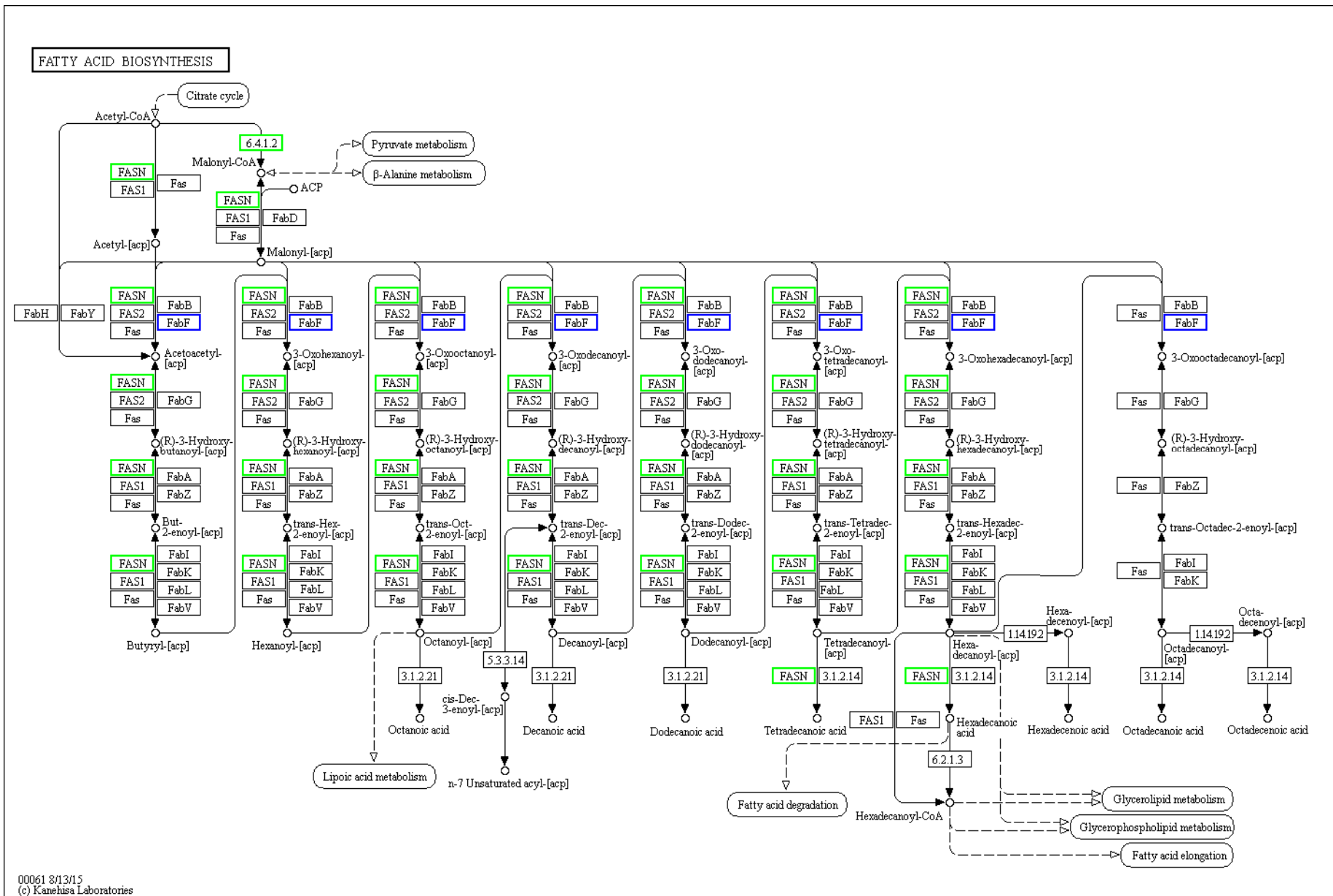
Figure Legends

Supplementary Table 1. Proteins identified in female adult *Cx. pipiens pallens* using iTRAQ.

Supplementary Table 2. PRM verification analysis of overwintering diapause-related proteins.

Supplementary figure 1. Protein overview of complete metabolism in biological systems in BW vs. SUM, map0110075-77
 Green indicates metabolic pathways in which proteins are downregulated; red indicates metabolic pathways in which proteins are upregulated; blue indicates metabolic pathways in which proteins were quantified but do not participate in up/down regulation.

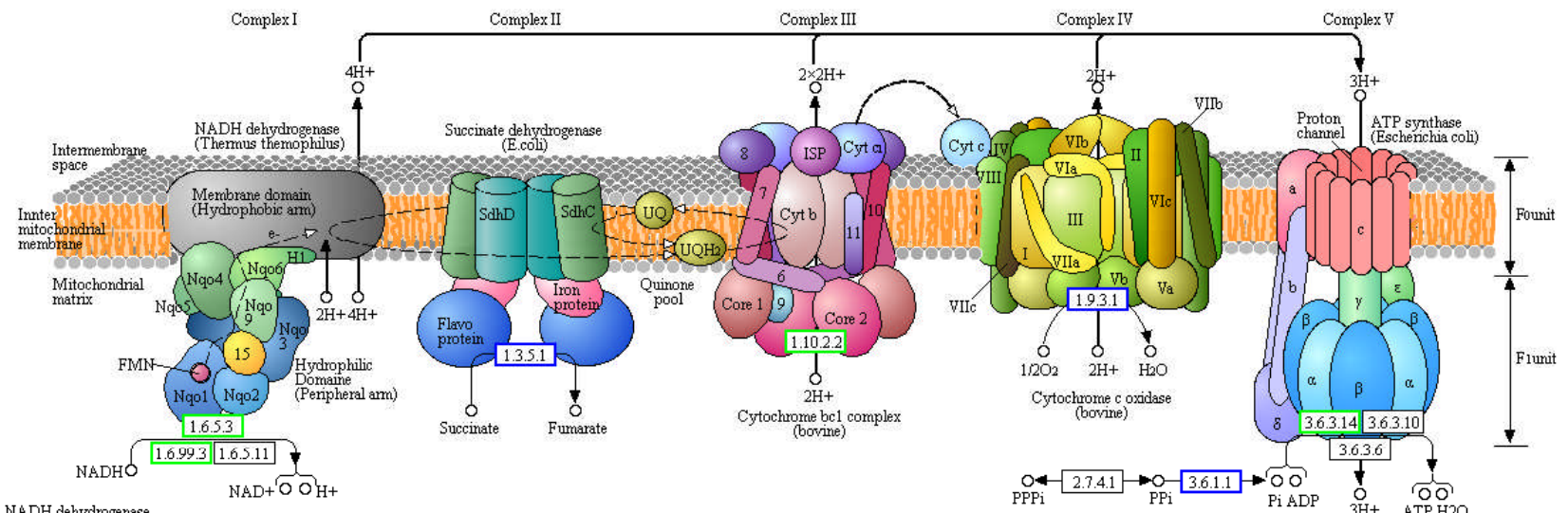




Supplementary figure 2. Annotated KEGG map for fatty acid biosynthesis in BW vs. SUM, map00061⁷⁵⁻⁷⁷

The blue border belongs to the background protein, and the black border indicates the protein was not identified in this experiment. The red/green colour in the figure marks the differentially expressed proteins detected in this experiment, in which red represents upregulated proteins and green represents downregulated proteins. Half red and half green indicates the protein is both upregulated and downregulated (the same meaning as in this manuscript).

OXIDATIVE PHOSPHORYLATION



NADH dehydrogenase

E	ND1	ND2	ND3	ND4	ND4L	ND5	ND6										
E	Ndufs1	Ndufs2	Ndufs3	Ndufs4	Ndufs5	Ndufs6	Ndufs7	Ndufs8	Ndufv1	Ndufv2	Ndufv3						
B/A	NuoA	NuoB	NuoC	NuoD	NuoE	NuoF	NuoG	NuoH	NuoI	NuoJ	NuoK	NuoL	NuoM	NuoN			
B/A	NdhC	NdhK	NdhJ	NdhH	NdhA	NdhI	NdhG	NdhE	NdhF	NdhD	NdhB	NdhL	NdhM	NdhN	HoxE	HoxF	HoxU
E	Ndufa1	Ndufa2	Ndufa3	Ndufa4	Ndufa5	Ndufa6	Ndufa7	Ndufa8	Ndufa9	Ndufa10	Ndufab1	Ndufa11	Ndufa12	Ndufa13			
E	Ndufb1	Ndufb2	Ndufb3	Ndufb4	Ndufb5	Ndufb6	Ndufb7	Ndufb8	Ndufb9	Ndufb10	Ndufb11	Ndufc1	Ndufc2				

Succinate dehydrogenase / Fumarate reductase

E	SDHC	SDHD	SDHA	SDHB				
B/A	SdhC	SdhD	SdhA	SdhB	FrdA	FrdB	FrdC	FrdD

Cytochrome c reductase

E/B/A	ISP	Cyt b	Cyt 1				
E	COR1	QCR2	QCR6	QCR7	QCR8	QCR9	QCR10

Cytochrome c oxidase

E	COX10	COX3	COX1	COX2	COX4	COX5A	COX5B	COX6A	COX6B	COX6C	COX7A	COX7B	COX7C	COX8	COX11	COX15	COX17
B/A	CyoE	CyoD	CyoC	CyoB	CyoA	CoxD	CoxC	CoxA	CoxB	QoxD	QoxC	QoxB	QoxA	CydA	CydB		

Cytochrome c oxidase, cbb3-type

B	I	II	IV	III
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Cytochrome bd complex

B/A	CydA	CydB
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F-type ATPase (Bacteria)

alpha	beta	gamma	delta	epsilon
a	b	c		

F-type ATPase (Eukaryotes)

alpha	beta	gamma	delta	epsilon	
OSCP	a	b	c	d	e
f	g	f6/h	j	k	8

V/A-type ATPase (Bacteria, Archaeas)

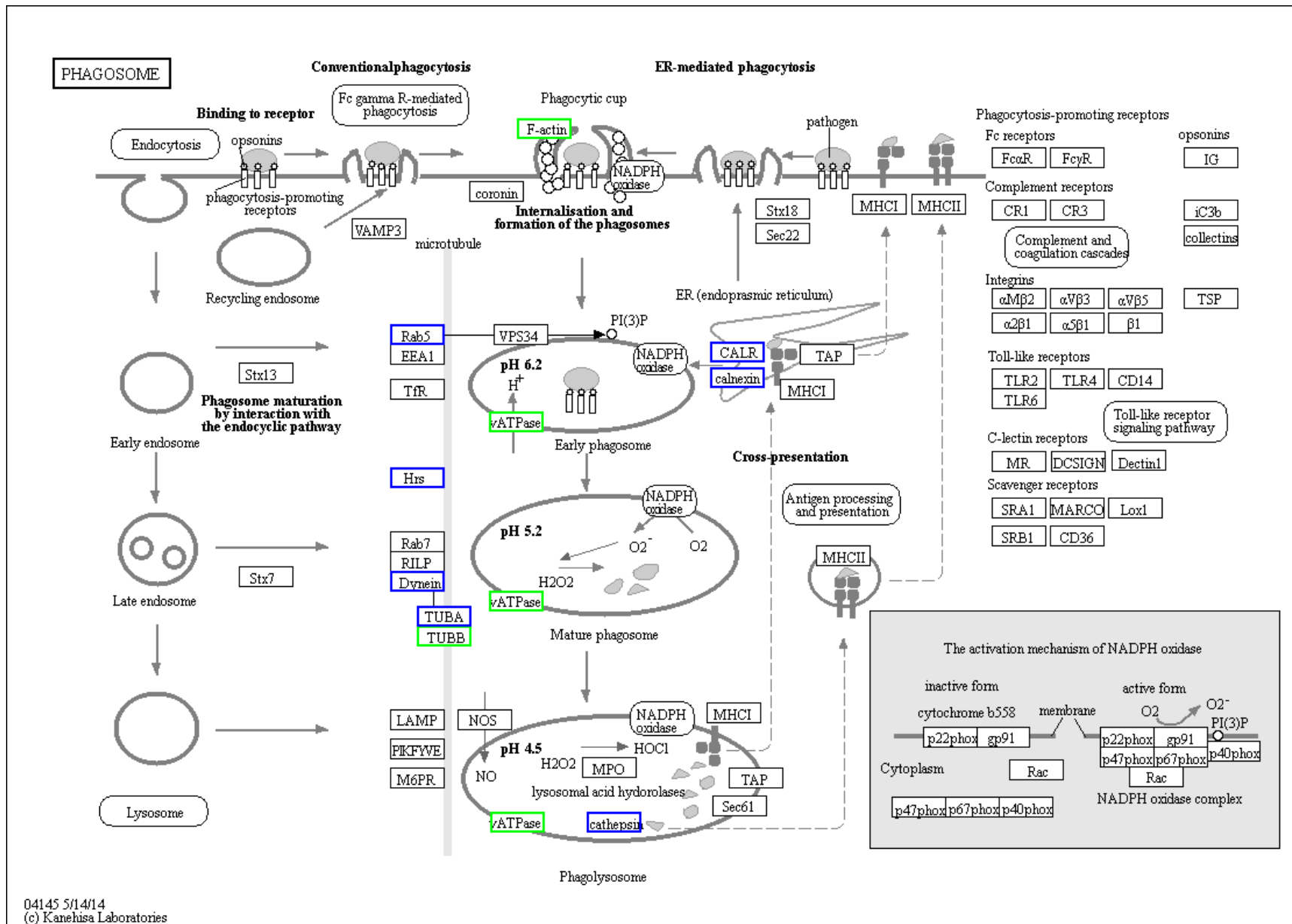
A	B	C	D	E	F	G/H
I	K					

V-type ATPase (Eukaryotes)

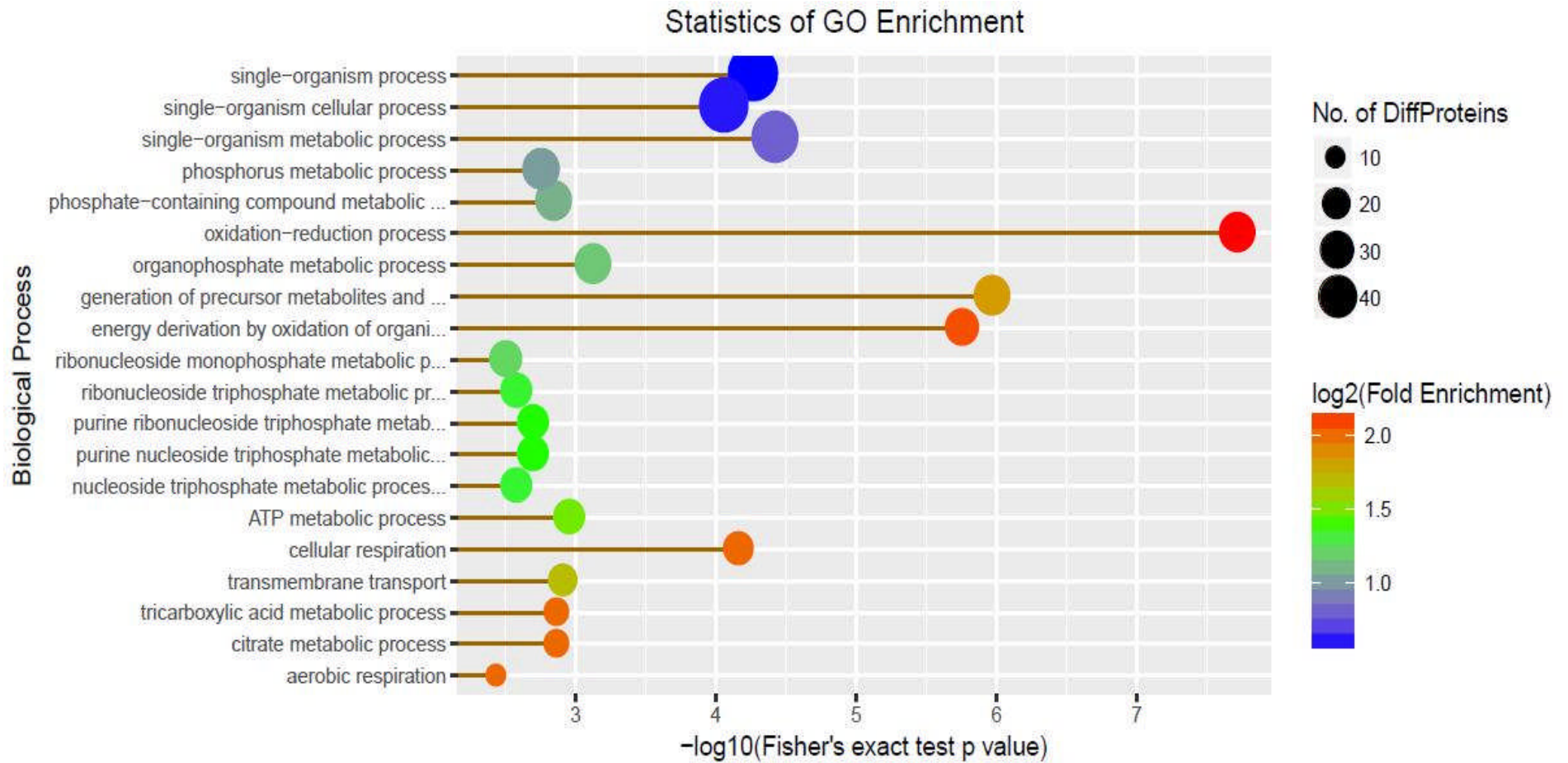
A	B	C	D	E	F	G	H
a	c	d	e	S1			

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Supplementary figure 3. Annotated KEGG map for oxidative phosphorylation in BW vs. SUM, map00190⁷⁵⁻⁷⁷

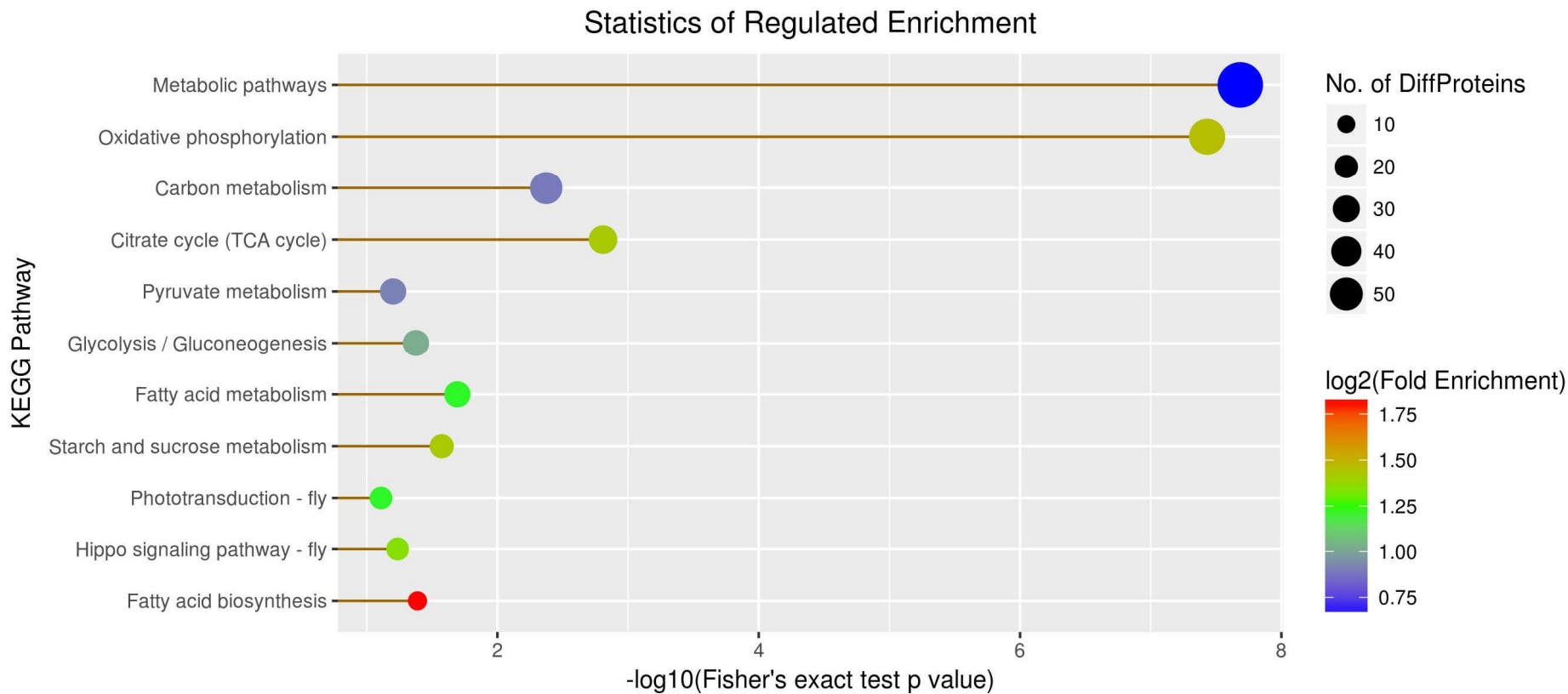


Supplementary figure 5. Annotated KEGG map for phagosomes in BW vs. SUM, map04145⁷⁵⁻⁷⁷



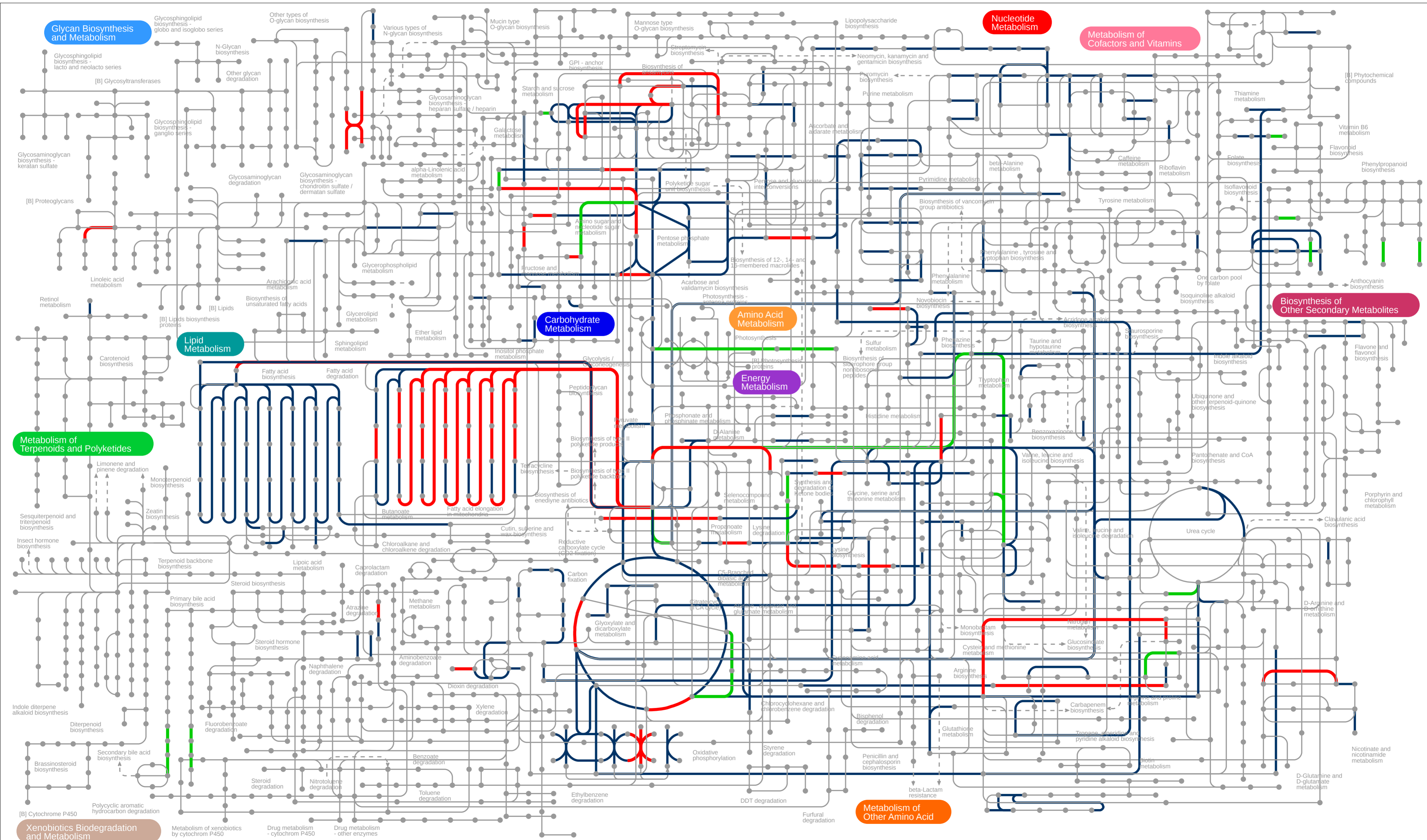
Supplementary figure 6. Visualization of significantly enriched GO terms in AW vs. BW

Upregulated proteins in the biological process category according to Fisher's exact test. The Number of Diffproteins is the number of differentially expressed proteins enriched in GO terms; the GO enrichment fold is shown on the \log_2 scale in a colour gradient.

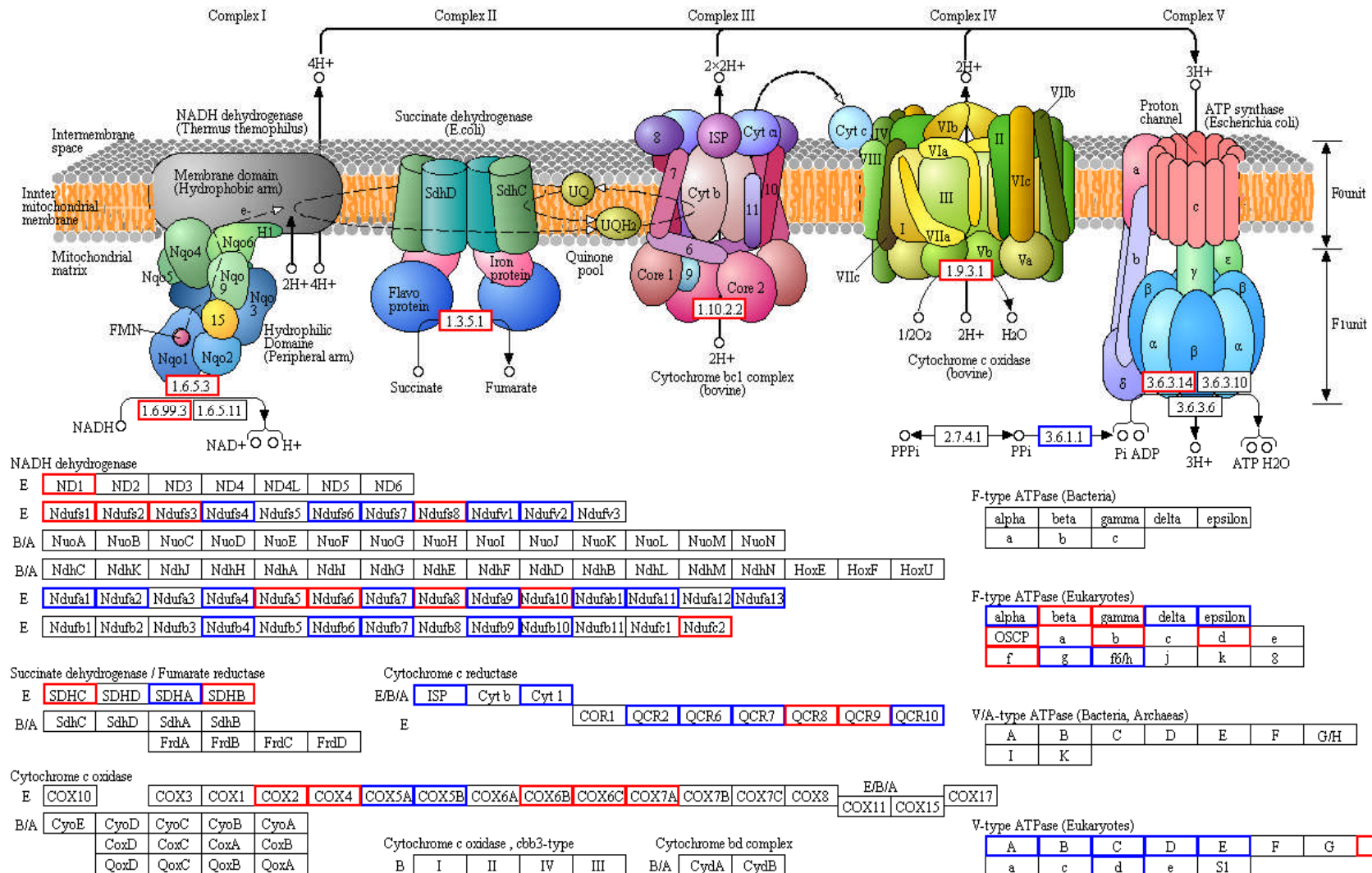


Supplementary figure 7. KEGG pathway enrichment analysis of differentially expressed proteins in AW vs. BW. The pathway enrichment statistical analysis was performed by Fisher's exact test. When the p value is 0.05, it is considered that there is significant enrichment in this KEGG pathway.

Supplementary figure 8. Protein overview of complete metabolism in biological systems in AW vs. BW, map01100⁷⁵⁻⁷⁷

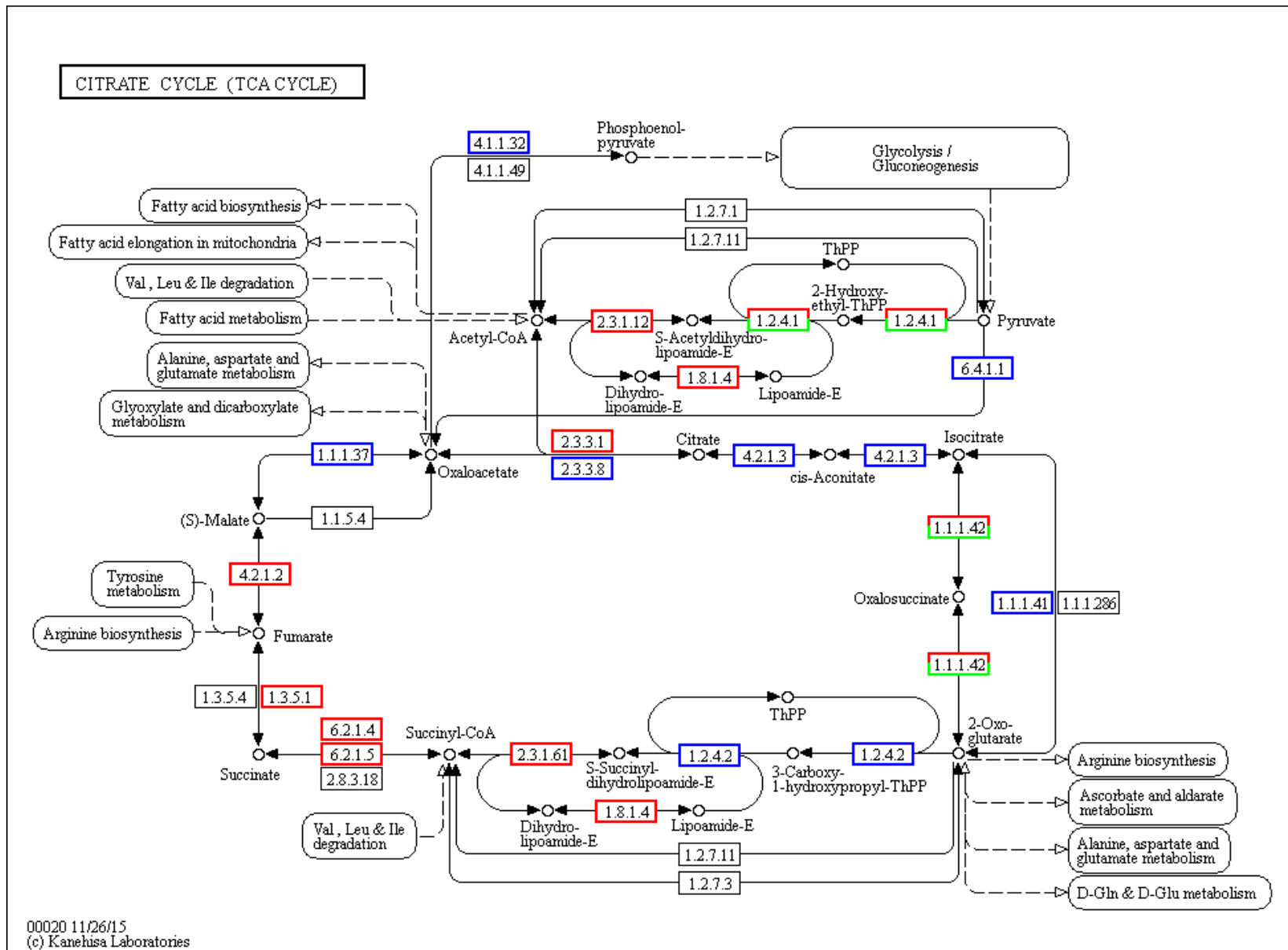


OXIDATIVE PHOSPHORYLATION

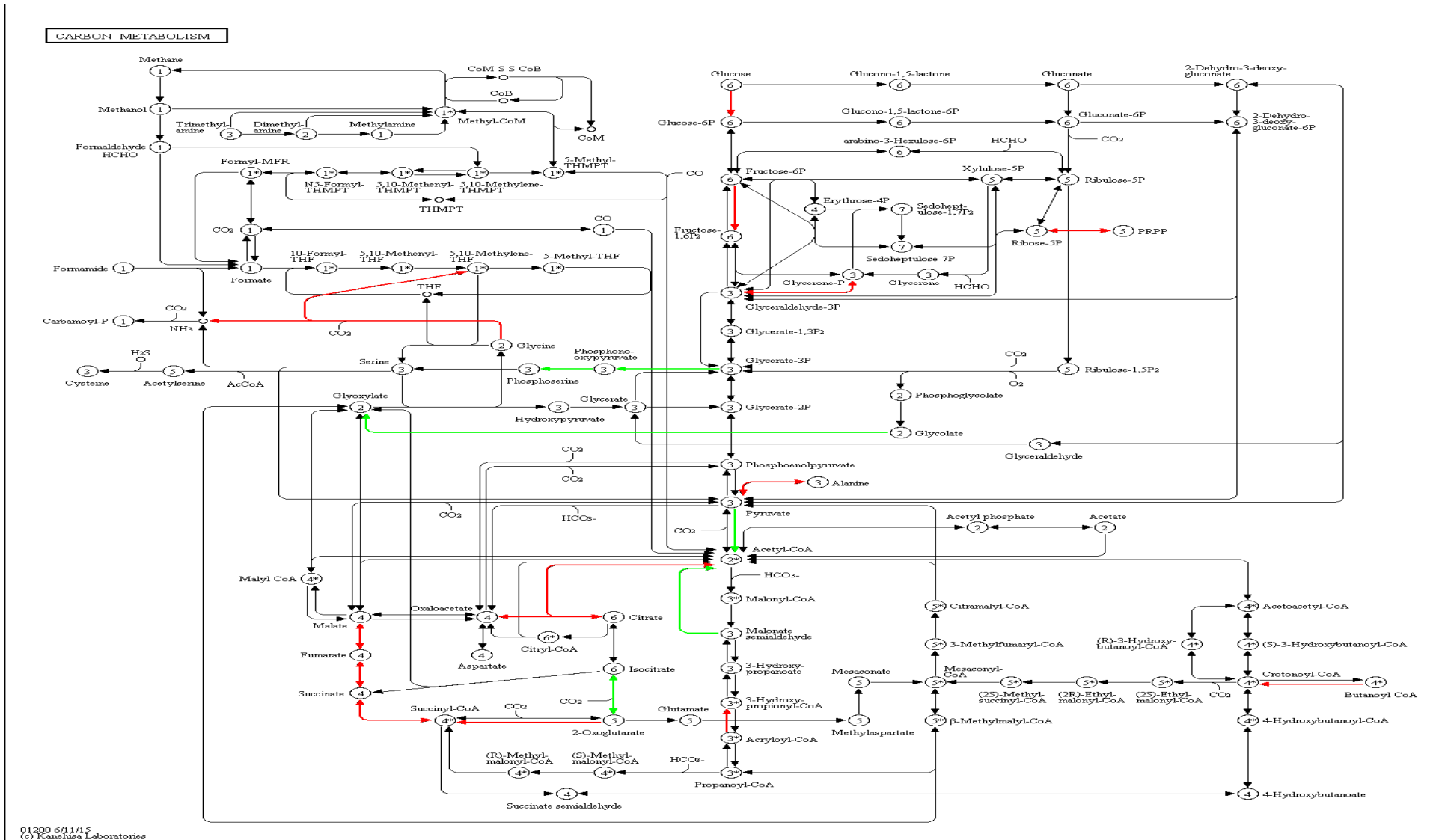


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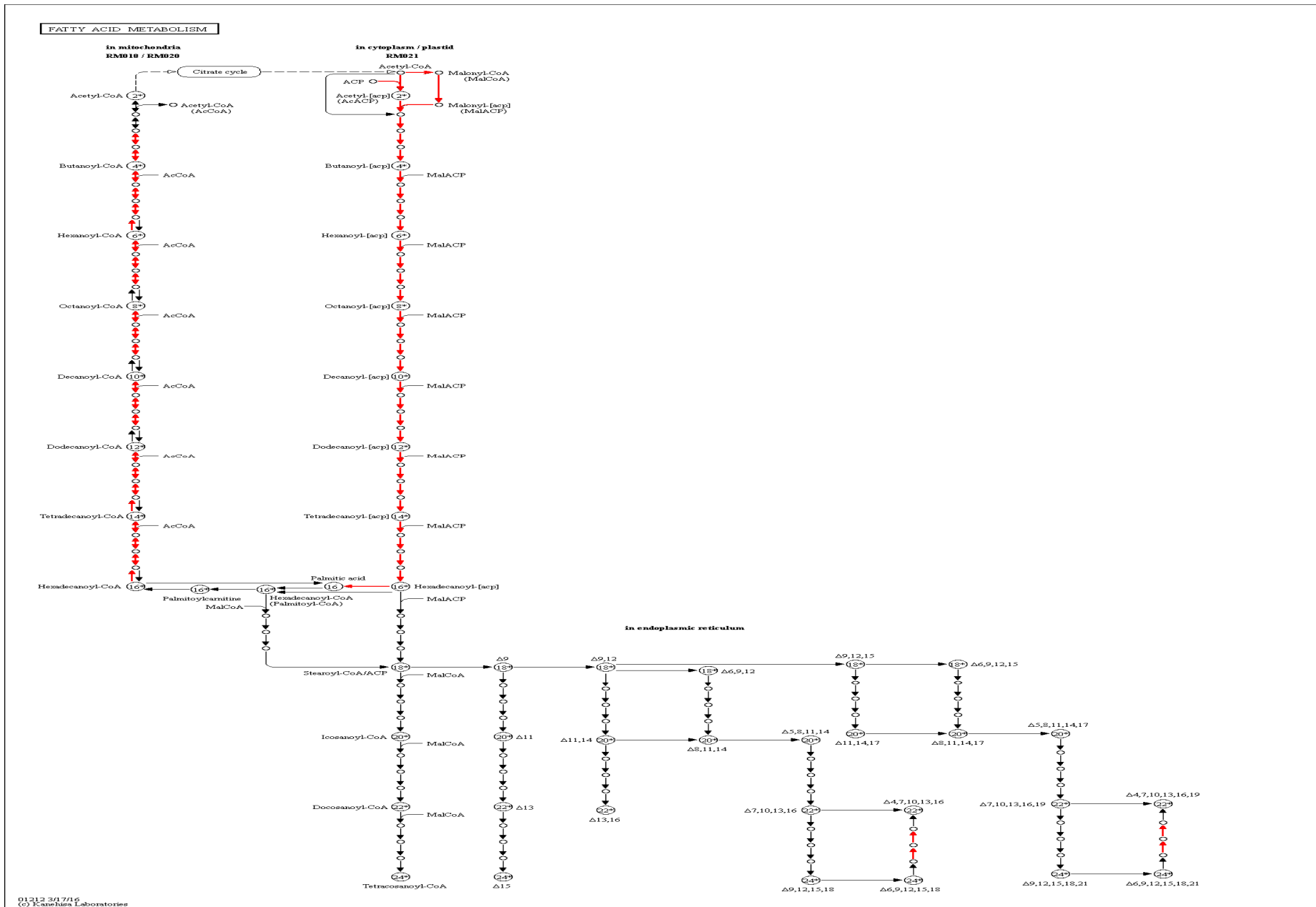
Supplementary figure 9. Annotated KEGG map for oxidative phosphorylation in AW vs. BW, map00190⁷⁵⁻⁷⁷



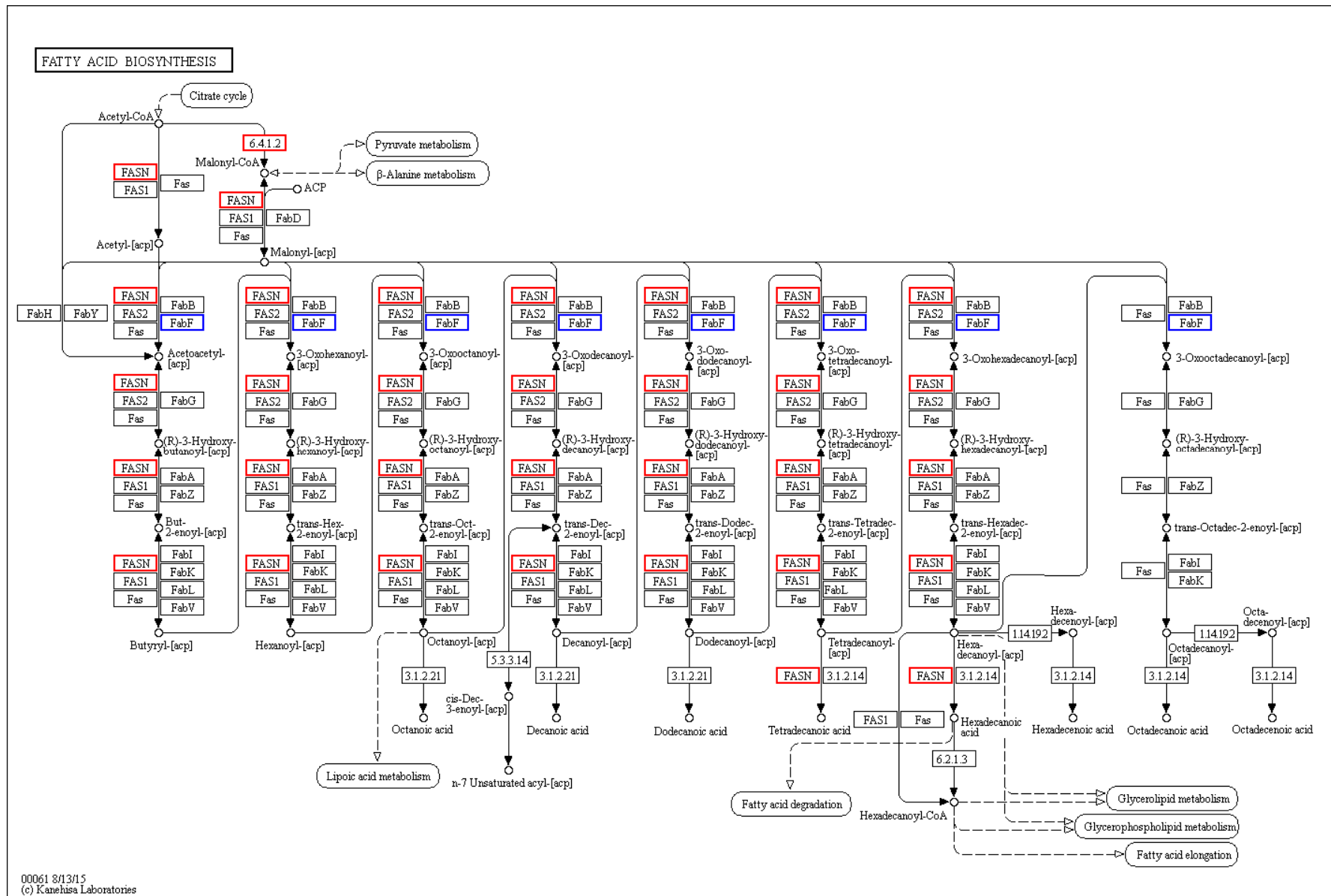
Supplementary figure 10. Annotated KEGG map for citrate cycle (TCA cycle) in AW vs. BW, map00020⁷⁵⁻⁷⁷



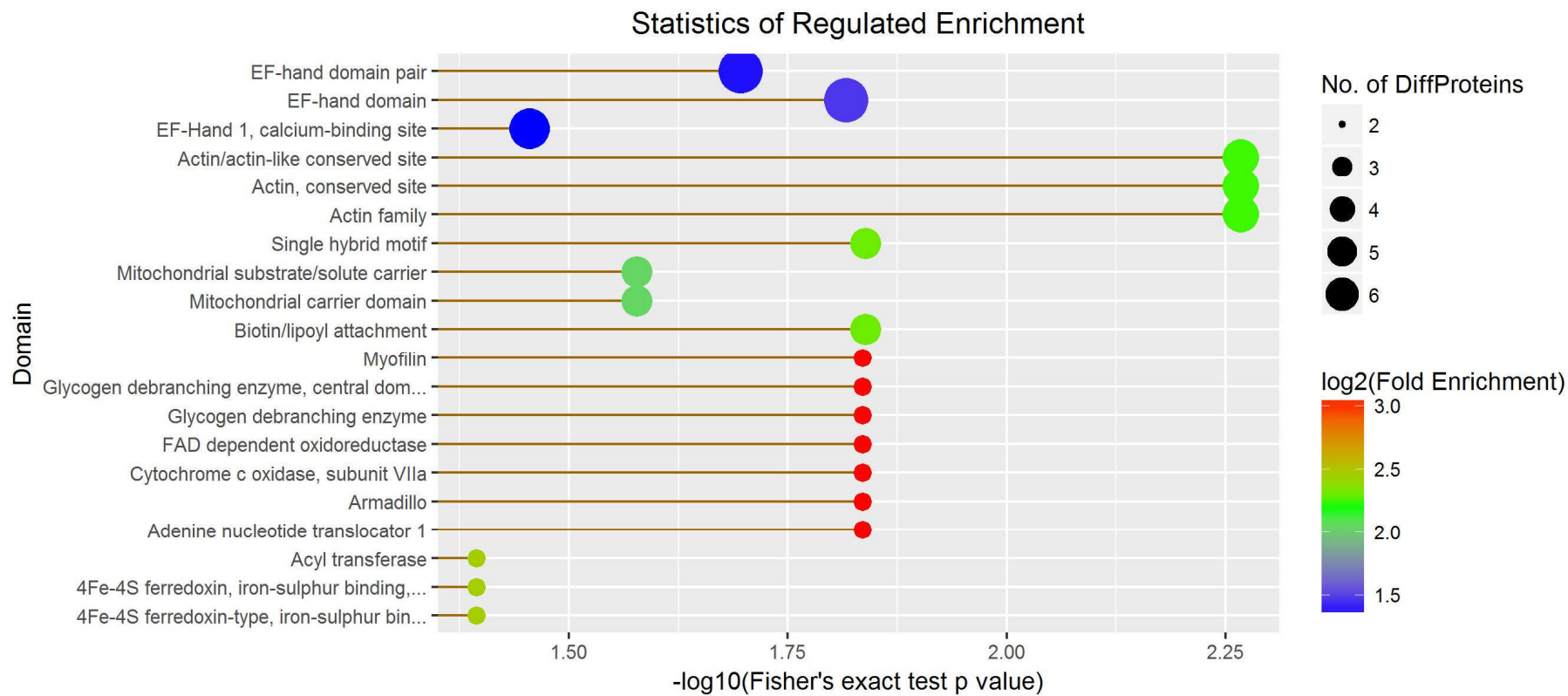
Supplementary figure 11. Annotated KEGG map for carbon metabolism in AW vs. BW, map01200⁷⁵⁻⁷⁷



Supplementary figure 12. Annotated KEGG map for fatty acid metabolism in AW vs. BW, map01212⁷⁵⁻⁷⁷

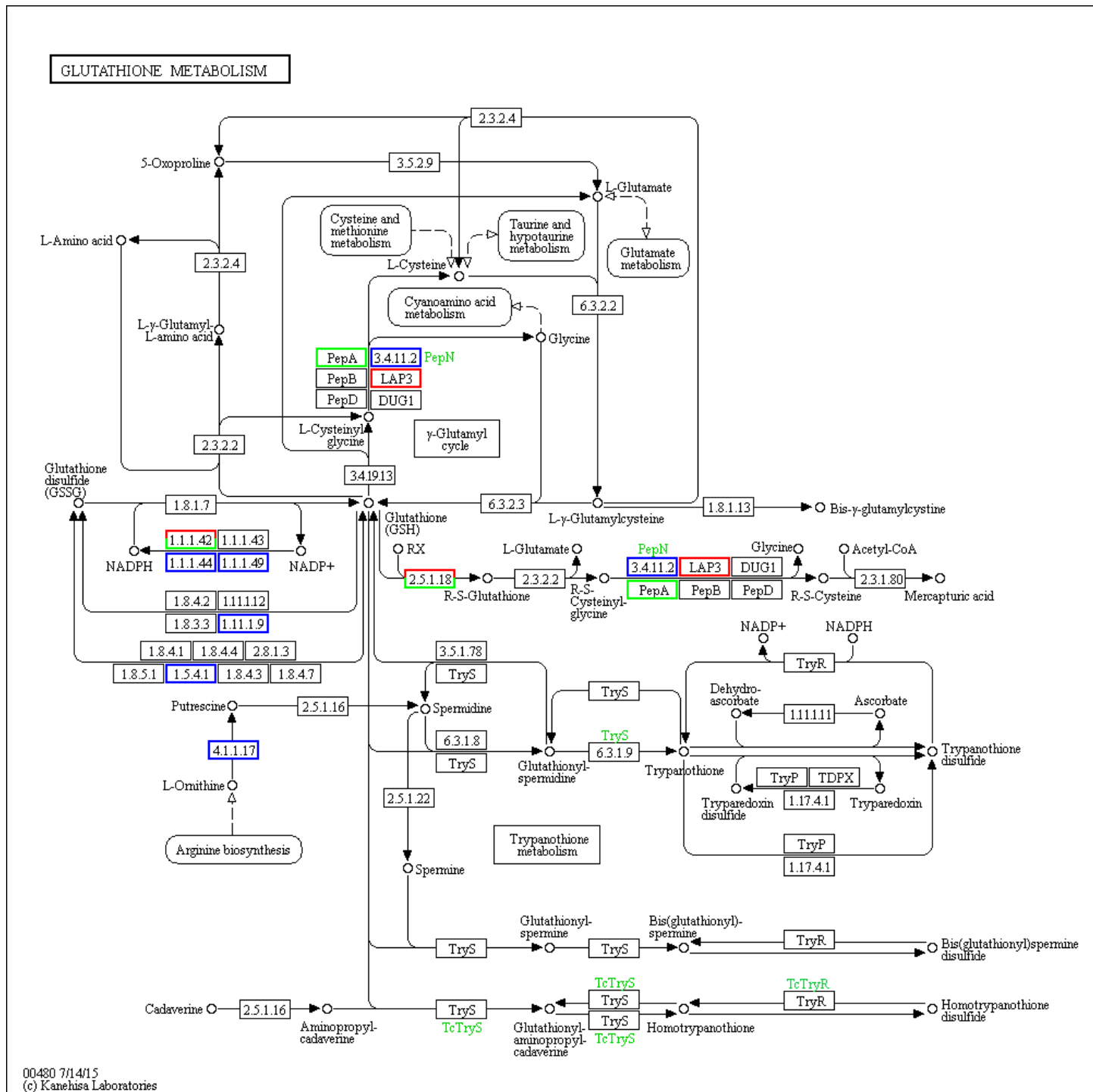


Supplementary figure 14. Annotated KEGG map for fatty acid biosynthesis in AW vs. BW, map00061⁷⁵⁻⁷⁷



Supplementary figure 16. Visualization of significantly enriched Domain in AW vs. BW.

Upregulated proteins in functional categorizations of proteins differently expressed according to Fisher's exact test. The Number of Diffproteins is the number of differentially expressed proteins enriched in the Domain; the Domain enriched fold is shown on a log2 scale in a colour gradient.



Supplementary figure 17. Annotated KEGG map for glutathione metabolism in AW vs. BW, map00480⁷⁵⁻⁷⁷