Online Data Supplement

Protein O-GlcNAcylation is essential for the maintenance of renal energy homeostasis and function via lipolysis during fasting and diabetes

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Contents

Supplemental Table 1. List of antibodies used in this study.

Antibody	Company	Catalog No.
Cre recombinase	Cell Signaling	#12830
O-GlcNAc transferase (Ogt)	Abcam	#ab96718
RL2 (O-GlcNAcylation)	Abcam	#ab2739
Cleaved caspase 3	Cell Signaling	#9661
Poly（ADP-ribose）polymerase (PARP)	Cell Signaling	#9542
β actin	Sigma Aldrich	#123M4876
Carboxylesterase 1 (CES1)	Abcam	#ab45957
Phosphorylated hormone sensitive lipase (pHSL^{ser563})	Cell Signaling	#4139
Hormone sensitive lipase (HSL)	Cell Signaling	#4107
Adipose triglyceride lipase (ATGL)	Cell Signaling	#2138
Perilipin	Cell Signaling	#9349
Peroxisome proliferator-activated receptor-α (PPARα)	Santa Cruz	#sc-9000
Farnesoid X receptor (FXR)	Abcam	#ab28480
Fibronectin	Millipore	#AB2033
F4/80	BIO-RAD	#MCA497GA
Lipoprotein lipase (LPL)	Abcam	#ab21356
Lotus Tetragonolobus lectin (LTL)	Vector Laboratories	#B-1325

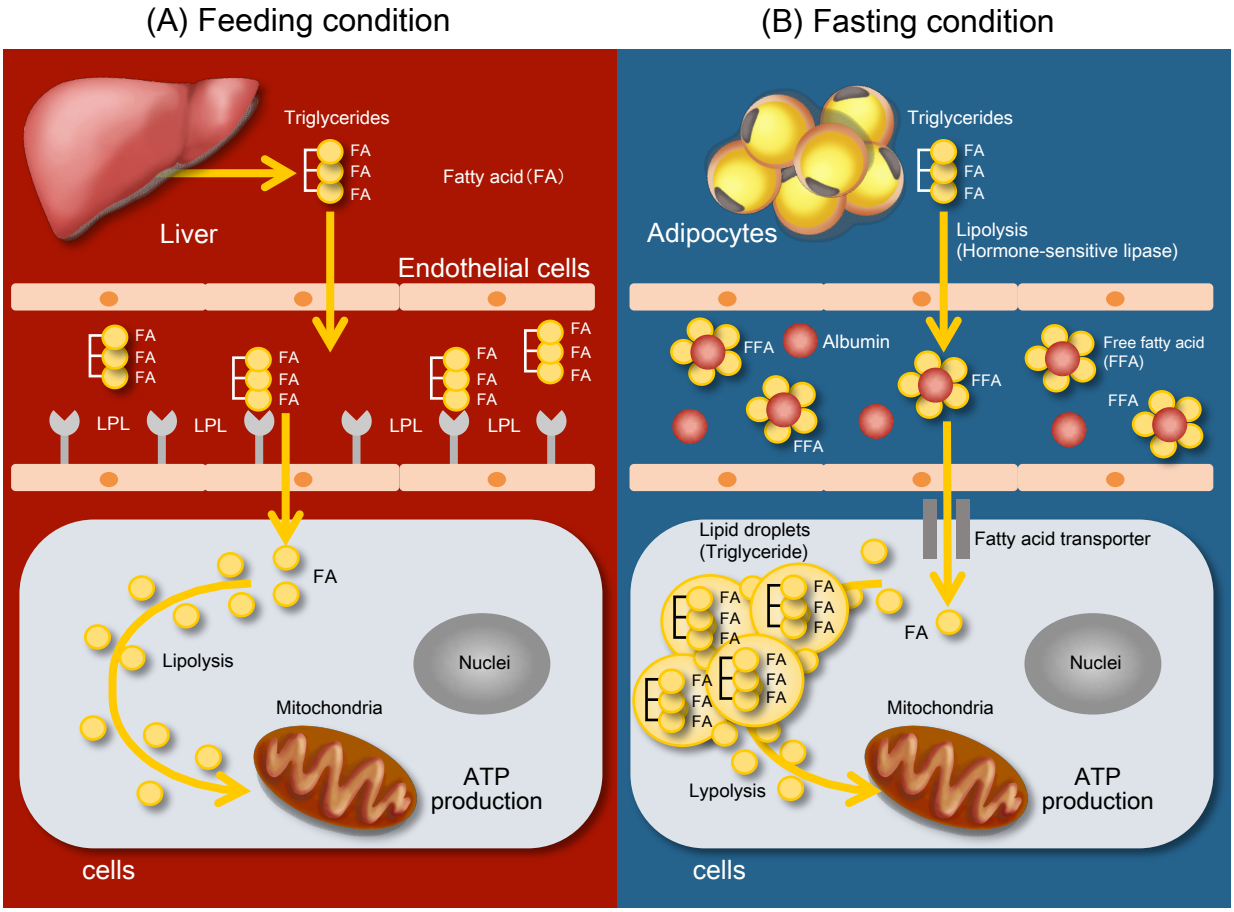
Supplemental Table 2. List of primer sets used in this study.

mRNA	Accession No.	Forward	Reverse
ATP1b1	NM_009721.6	5’-tgtgcaggttcaagcttgac-3’	5’-ttcggtttgaagcccaacac-3’
NaPi-IIa	NM_011392.2	5’-taactggctgtctgttctggtc-3’	5’-tgaaggaagcaaccacaagc-3’
URAT	NM_009203.3	5’-ttggacccgatgttcttctgg-3’	5’-aagctgccattgaggttgtc-3’
SGLT2	NM_133254.4	5’-attgtctcgggctggtattgg-3’	5’-acaagatgcacccagctttg-3’
NHE3	NM_001081060.1	5’-atcacctttgcggcatctg-3’	5’-actggccagcatcttcatagtg-3’
β actin	NM_007393.5	5’-cgtgcgtgacatcaaagagaa-3’	5’-tggatgccacaggattccat-3’
OAT1	NM_008766.3	5’-tggtttgccactagctttgc-3’	5’-aggaagcacacaaacttggc-3’
LPL	NM_008509.2	5’-gcccagcaacattatccagt-3’	5’-ggtcagacttcctgctacgc-3’
GPIHBP1	NM_026730.2	5’-tgcaatcagacacagagctg-3’	5’-acaagtgaagaagcggttcc-3’
CES1	NM_021456.4	5’-cttggatctctgaggtttgctc-3’	5’-gggttttggtagcacaaagg-3’

Supplemental Table 3. Fold changes in expression levels of proteins associated with fatty acid metabolism in the proteomic analysis using renal cortex samples obtained from $Ogt^{y/f}$ mice (control) and PTEC-$Ogt^{y/-}$ mice (Knockout).

Fold change	Highest condition	Lowest condition	Description	Function
10.3	Control	Knockout	Carboxylesterase 1C	Triglyceride esterase
3.6	Control	Knockout	Acylcarnitine hydrolase (Carboxylesterase 2)	Carboxylic ester hydrolase
3.3	Knockout	Control	Acyl-coenzyme A thioesterase 11	Fatty acid synthase
2.6	Control	Knockout	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	Beta oxidation
2.6	Knockout	Control	Perilipin-2	Lipid droplet
2.2	Control	Knockout	Acyl-coenzyme A thioesterase 1	Fatty acid synthase
2.1	Knockout	Control	Acyl-CoA synthetase family member 2, mitochondrial	Fatty acid synthase
2.1	Control	Knockout	Very-long-chain enoyl-CoA reductase	Fatty acid synthase
2.0	Control	Knockout	ATP synthase subunit epsilon, mitochondrial	Mitochondria
2.0	Knockout	Control	Acyl-coenzyme A thioesterase 9, mitochondrial	Fatty acid synthase
2.0	Control	Knockout	Enoyl-CoA hydratase domain-containing protein 3, mitochondrial	Beta oxidation
2.0	Knockout	Control	Mitochondrial fission 1 protein	Mitochondria fission
1.9	Knockout	Control	Glutamate dehydrogenase 1, mitochondrial	Gluconeogenesis
1.9	Control	Knockout	Carboxylesterase 1D	Triglyceride esterase
1.9	Control	Knockout	3-ketoacyl-CoA thiolase B, peroxisomal	Beta oxidation
1.8	Control	Knockout	Peroxisomal acyl-coenzyme A oxidase 1	Beta oxidation
1.8	Knockout	Control	Enoyl-[acyl-carrier-protein] reductase, mitochondrial	Fatty acid synthase
1.8	Control	Knockout	Carboxylesterase 1F	Triglyceride esterase
1.8	Control	Knockout	ATPase inhibitor, mitochondrial	Mitochondria
1.7	Knockout	Control	5'-AMP-activated protein kinase catalytic subunit alpha-1	AMPK
1.7	Knockout	Control	Elongation factor Tu, mitochondrial	Mitochondria
1.7	Knockout	Control	Glycerol-3-phosphate dehydrogenase, mitochondrial	Glycerol-3-phosphate
1.7	Knockout	Control	Hydroxymethylglutaryl-CoA lyase, mitochondrial	Ketone metabolism
1.7	Knockout	Control	Acyl-coenzyme A synthetase ACSM1, mitochondrial	Beta oxidation
1.7	Knockout	Control	Acyl-CoA dehydrogenase family member 10	Beta oxidation
1.7	Control	Knockout	Carboxylesterase 1E	Triglyceride esterase
1.7	Knockout	Control	Glutaminase kidney isoform, mitochondrial	Gluconeogenesis
1.6	Knockout	Control	Acyl-coenzyme A thioesterase 8	Fatty acid synthase
1.6	Knockout	Control	Acyl-coenzyme A thioesterase 13	Fatty acid synthase
1.6	Knockout	Control	Peroxisomal acyl-coenzyme A oxidase 2	Beta oxidation
1.6	Control	Knockout	Very long-chain acyl-CoA synthetase	Beta oxidation
1.6	Knockout	Control	Acyl-CoA synthetase family member 3, mitochondrial	Beta oxidation
1.6	Knockout	Control	Cytochrome c oxidase assembly protein COX15 homolog	Mitochondria
1.6	Knockout	Control	Acyl-coenzyme A thioesterase	Fatty acid synthase
1.6	Knockout	Control	Cytochrome c oxidase subunit 7A1, mitochondrial	Mitochondria
1.6	Knockout	Control	Cytochrome c oxidase subunit 7A-related protein, mitochondrial	Mitochondria
1.6	Knockout	Control	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	Glycerol-3-phosphate
1.6	Knockout	Control	Acyl-CoA dehydrogenase family member 9, mitochondrial	Beta oxidation
1.5	Control	Knockout	Phosphoglycerate kinase 1	Gluconeogenesis/glycolysis
1.5	Knockout	Control	Peroxisomal acyl-coenzyme A oxidase 3	Fatty acid synthase
1.5	Control	Knockout	Fatty acid-binding protein, epidermal	Fatty acid-binding protein
1.5	Knockout	Control	Enoyl-CoA hydratase, mitochondrial	Beta oxidation
1.5	Control	Knockout	Enoyl-CoA delta isomerase 3, peroxisomall	Beta oxidation
1.5	Knockout	Control	Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial	Ketolysis

Supplemental Figure 1



Supplemental Figure 1. Fatty acid transport into cells during feeding and fasting.
(A) During the fed state, most cells can utilize fatty acids in an lipoprotein lipase (LPL)-dependent manner. FAs are de-esterified by LPL on the endothelial surface, enter cells, and are metabolized to yield ATP in mitochondria. (B) During the fasting state, fatty acids released from adipocytes circulate bound to albumin, and are transported into cells. After this, FAs are esterified and stored in intracellular lipid droplets. Finally, stored FAs in the lipid droplet can be used to generate ATP following lipolysis.

Supplemental Figure 2

Figure 1B. Cre

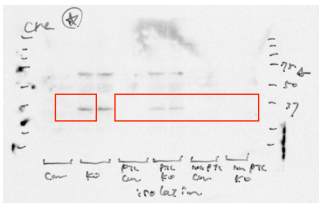


Figure 1B. RL2

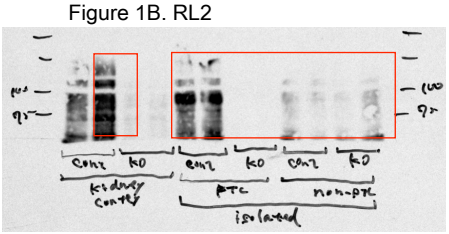


Figure 2E. Caspase 3

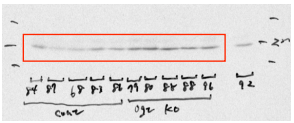


Figure 2E. β actin

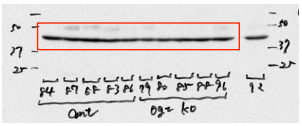


Figure 1B. Ogt

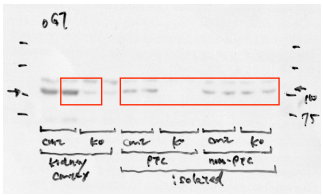


Figure 1B. β actin

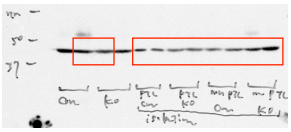


Figure 4B. LPL (MW: 53 kd)

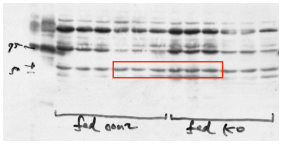


Figure 4D. IP:RL2 IB:LPL

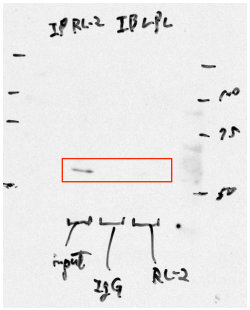


Figure 4J. Caspase 3

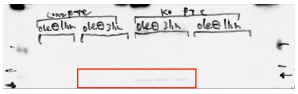


Figure 4J. β actin

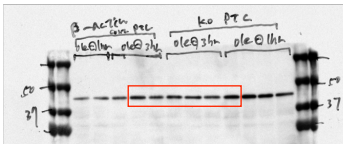


Figure 4J. PARP

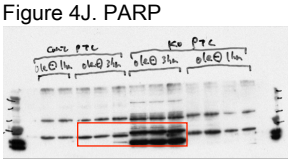


Figure 4B. β actin

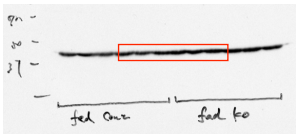


Figure 6C. CES1

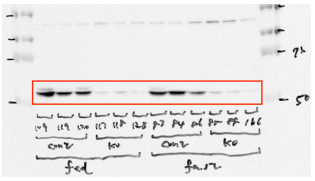


Figure 6H. Caspase 3

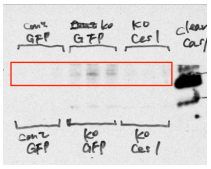


Figure 5B. pHSL (Ser563)

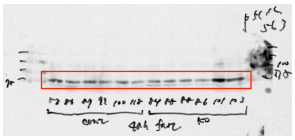


Figure 5B. ATGL

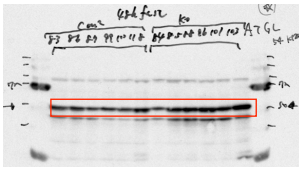


Figure 5B. HSL

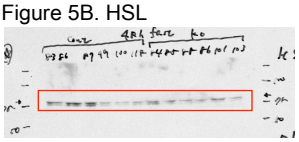


Figure 6C. β actin

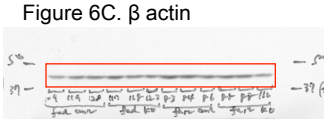


Figure 6H. β actin

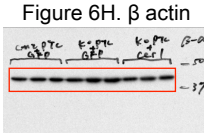


Figure 5B. Perilipin

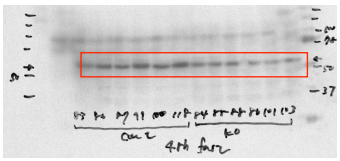


Figure 5B. β actin

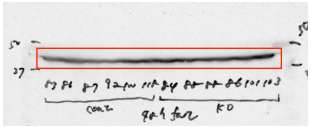


Figure 7C. IP:RL2 IB:FXR

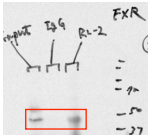


Figure 7C. IP:FXR IB:RL2

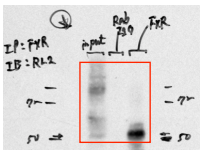


Figure 7D. IP:RL2 IB:Perilipin

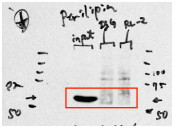


Figure 7D. IP:RL2 IB:CES1

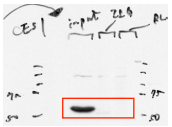


Figure 7D. IP:RL2 IB:ATGL

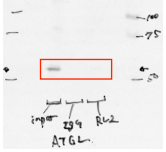


Figure 7D. IP:RL2 IB:HSL

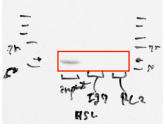
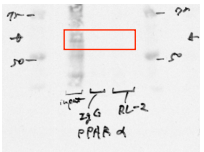


Figure 7D. IP:RL2 IB:PPARα



Supplemental Figure 2. Full gel scan images for the immmune blots of the indicated figure number. The areas indicated by the red box were used in the representative figures of the manuscript.