

Galectin-3 gene deletion results in defective adipose tissue maturation and impaired insulin sensitivity and glucose homeostasis

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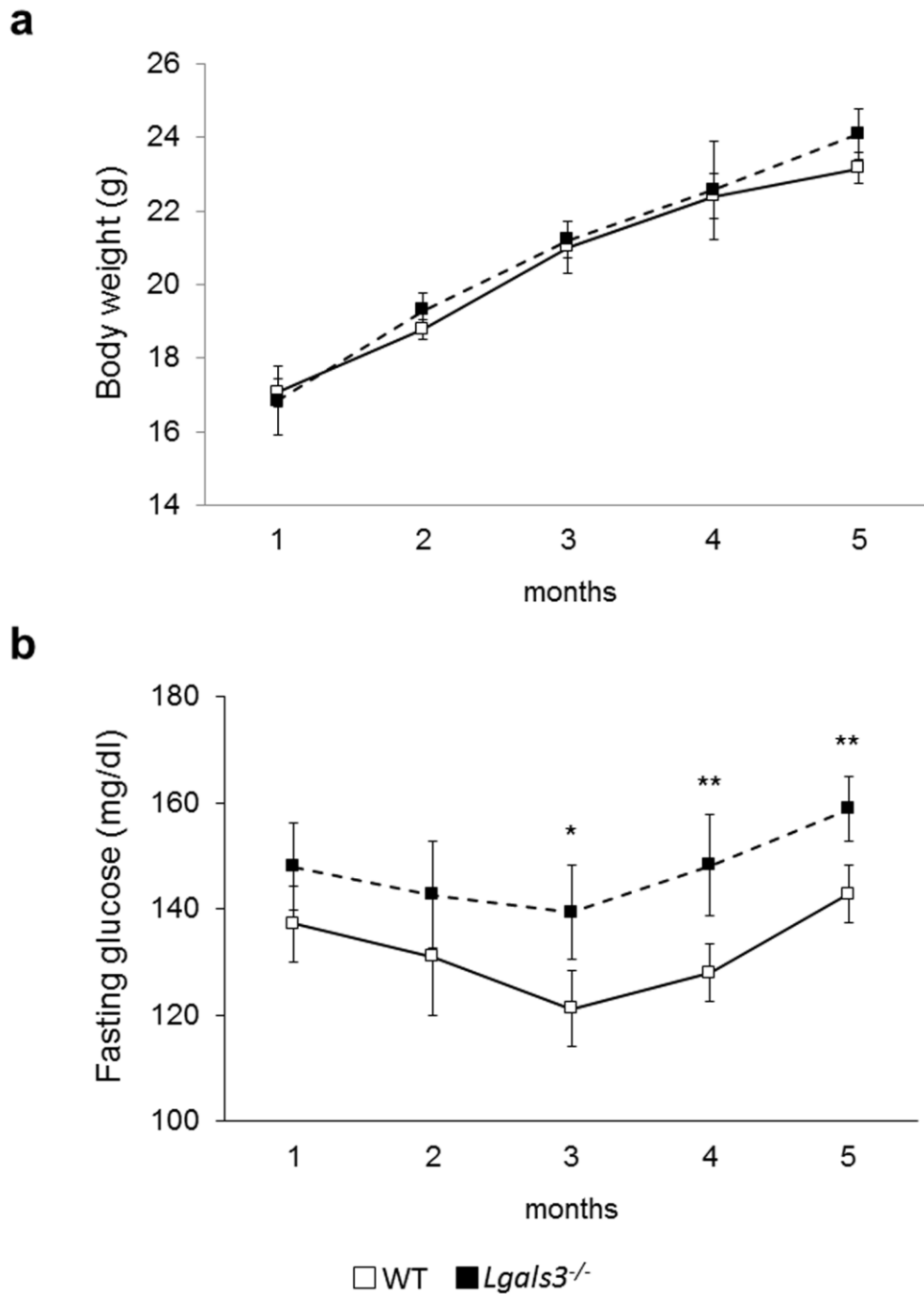
Supplementary Table 1. Primary and secondary antibody list used in Western blot and immunohistochemistry.

Primary Antibody	Species	Dilution	Company	Catalog number
Akt 1/2/3	rabbit monoclonal	1:1,000	Abcam (Cambridge, UK)	AB 179463
Akt p	rabbit polyclonal	1:1,000	Abcam (Cambridge, UK)	AB 66138
Galectin-3	mouse monoclonal	1:5000	Abcam (Cambridge, UK)	AB 2785
β-actin	mouse monoclonal	1:10,000	Merck KGaA (Darmstadt, Germany)	A5441
F4/80	rat monoclonal	1:200	Novus Biologicals (Littleton, CO, USA)	NB 600-404
Perilipin-1	goat polyclonal	1:40	Abcam (Cambridge, UK)	AB 61682
Secondary Antibody	Species	Dilution	Company	Catalog number
HRP-conjugated anti rabbit Ig	goat	1:5,000	Dako (Glostrup, Denmark)	P0448
HRP-conjugated anti mouse Ig	goat	1:2,500	Dako (Glostrup, Denmark)	P0447
Anti-rat IgG H&L (Biotin)	goat	1:400	Abcam (Cambridge, UK)	AB 6844
Anti-goat IgG H&L (Biotin)	rabbit	1:500	Abcam (Cambridge, UK)	AB 6740

Supplementary Table 2. TaqMan Assays used in quantitative real time PCR.

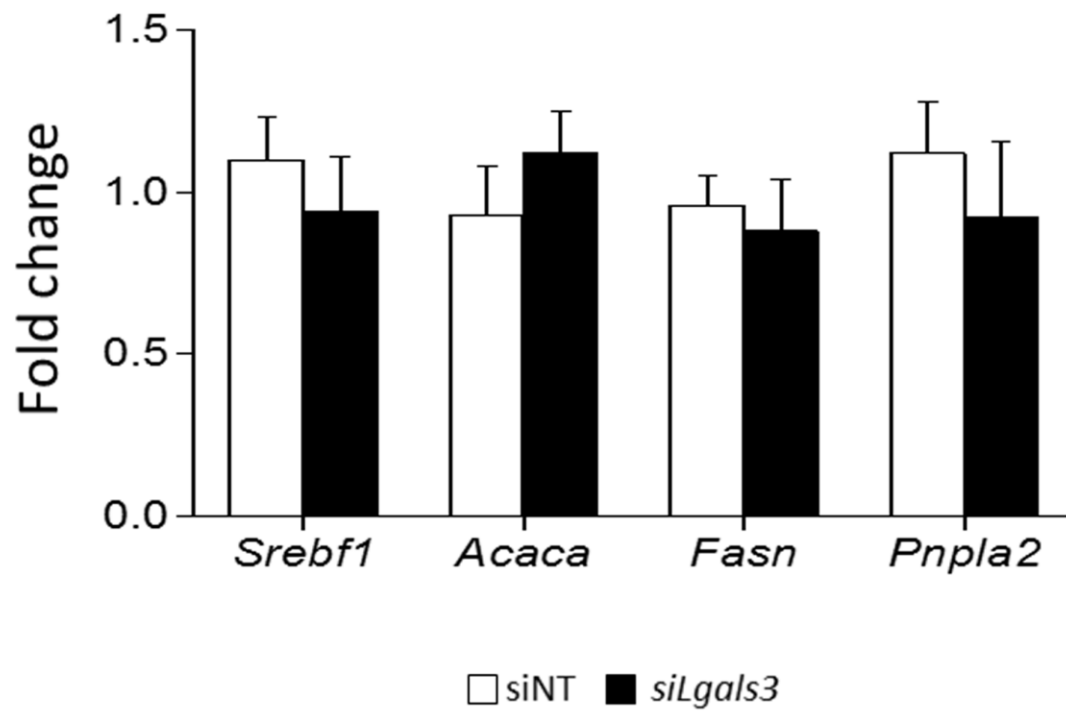
Mouse TaqMan Assay	Code
<i>Lgals3</i>	Mm00802901_m1
<i>Dlk1</i>	Mm00494477_m1
<i>Cebpa</i>	Mm00514283_s1
<i>Cebpb</i>	Mm00843434_s1
<i>Pparg</i>	Mm01184322_m1
<i>Pnpla2</i>	Mm00503040_m1
<i>Srebf1</i>	Mm00550338_m1
<i>Acaca</i>	Mm01304257_m1
<i>Fasn</i>	Mm00662319_m1
<i>Fabp4</i>	Mm00445878_m1
<i>Lep</i>	Mm00434759_m1
<i>Adipoq</i>	Mm00456425_m1
<i>Cfd</i>	Mm01143935_g1
<i>Cidea</i>	Mm00432554_m1
<i>Ppara</i>	Mm00440939_m1
<i>Ucp1</i>	Mm01244861_m1
<i>Ccnd1</i>	Mm00432359_m1
<i>Insr</i>	Mm01211875_m1
<i>Irs1</i>	Mm01278327_m1
<i>Slc2a4</i>	Mm01245502_m1
<i>Tnfa</i>	Mm00443258_m1
<i>Il1b</i>	Mm00434228_m1
<i>Il6</i>	Mm00446190_m1
<i>Ccl2</i>	Mm00441242_m1
<i>Cd68</i>	Mm03047343_m1
<i>Adgre1</i>	Mm00802529_m1
<i>Fn1</i>	Mm01256744_m1
<i>Cola1a1</i>	Mm00801666_g1
<i>Cola4a1</i>	Mm01210125_m1
<i>Cola6a1</i>	Mm00711678_m1
<i>Actb</i>	Mm00607939_s1

Lgals3=galectin-3; *Dlk1*=Preadipocyte factor 1 or Delta Like Non-Canonical Notch Ligand; *Cebpa*=Ccaat-Enhancer-Binding Protein α ; *Cebpb*=Ccaat-Enhancer-Binding Protein β ; *Pparg*=peroxisome proliferator-activated receptor γ ; *Pnpla2*=Adipose Triglyceride Lipase or Patatin Like Phospholipase Domain Containing 2; *Srebf1*=Sterol Regulatory Element Binding Transcription Factor 1; *Acaca*=Acetyl-CoA Carboxylase α ; *Fasn*=Fatty Acid Synthase; *Fabp4*=Fatty Acid Binding Protein 4; *Lep*=Leptin; *Adipoq*=Adiponectin; *Cfd*=Adipsin or Complement Factor D; *Cidea*=Cell Death-Inducing DFFA-Like Effector A; *Ppara*=peroxisome proliferator-activated receptor α ; *Ucp1*=Uncoupling Protein 1; *Ccnd1*=Cyclin D1; *Insr*=Insulin Receptor; *Irs1*=Insulin Receptor Substrate 1; *Slc2a4*=Glucose Transporter Type 4 or Solute Carrier Family 2 Member 4; *Tnfa*=tumor necrosis factor α ; *Il1b*=interleukin 1 β ; *Il6*=interleukin 6; *Ccl2*= Monocyte Chemoattractant Protein-1 or C-C Motif Chemokine Ligand 2 (*Ccl2*); *Cd68*= Cluster of Differentiation 68; *Adgre1*=F4/80 or Adhesion G Protein-Coupled Receptor E1; *Fn1*=Fibronectin 1; *Cola1a1*=Collagen Type I Alpha 1 Chain; *Cola4a1*=Collagen Type IV Alpha 1 Chain; *Cola6a1*=Collagen Type VI Alpha 1 Chain; *Actb* = β -actin.

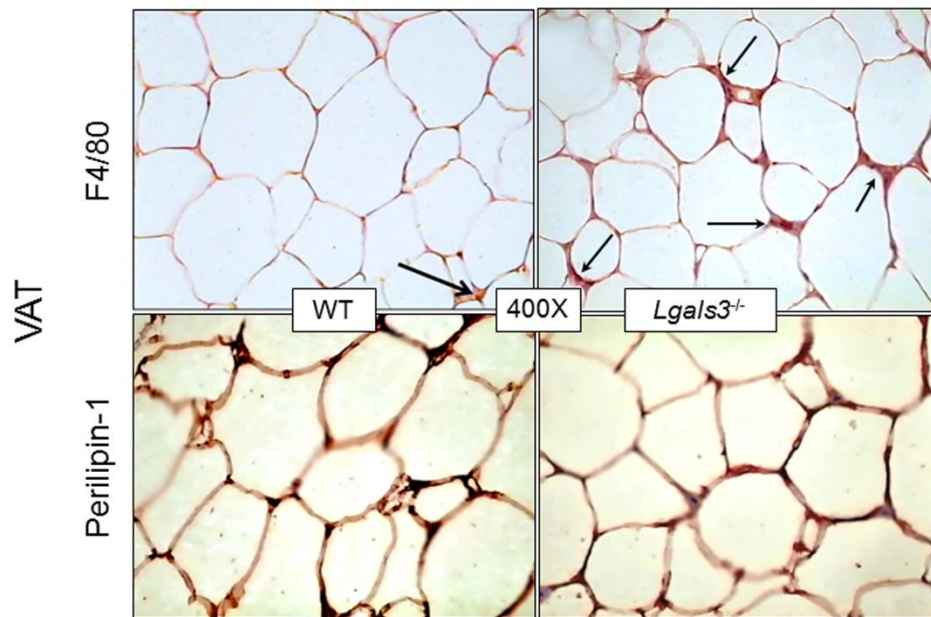


Supplementary Figure 1. Trends in body weight and fasting plasma glucose in WT and *Lgals3*^{-/-} mice.

Changes in body weight (**a**) and fasting plasma glucose levels (**b**) in WT and *Lgals3*^{-/-} mice throughout the study period. Values represent the mean±SD (n=17 per genotype). *P<0.05 or **P<0.01 versus WT.

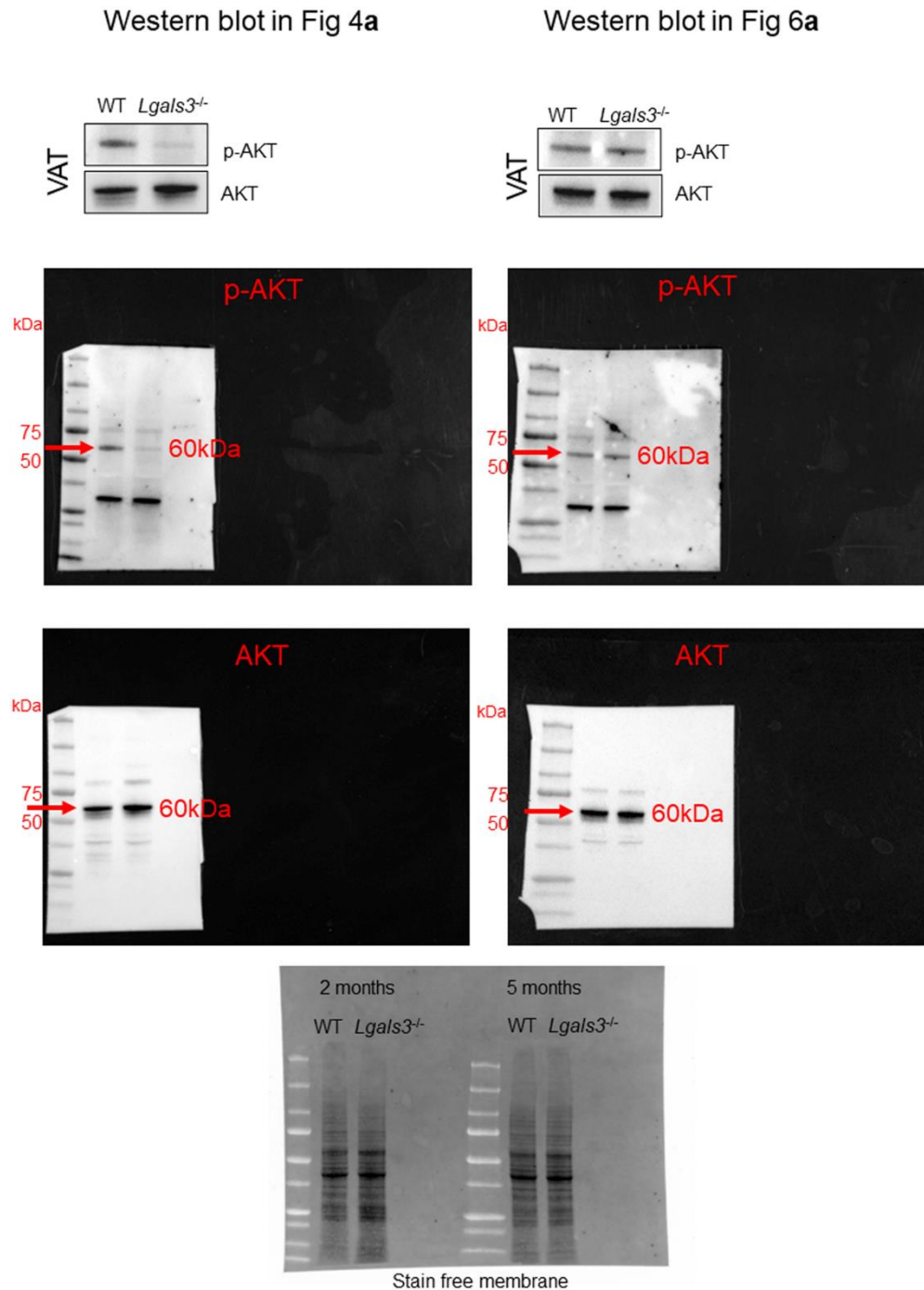


Supplementary Figure 2. Cellular lipid metabolism in 3T3-L1 silenced for *Lgals3*. RT-PCR analysis of genes involved in lipid metabolism (*Srebf1*, *Acaca*, *Fasn*, and *Pnpla2*) from 3T3-L1 siNT control and siLgals3 cells. *P<0.05, **P<0.01 or ***P<0.001 vs WT. siNT = non-target control.



Supplementary Figure 3. F4/80 and perilipin-1 staining in 2-month-old wild type and *Lgals3*^{-/-} mice.

Serial sections of VAT from representative 2-month-old WT and *Lgals3*^{-/-} mice immunolabelled for F4/80, a murine macrophage marker, and perilipin-1, a lipid droplet coating protein, using the antibodies reported in Supplementary Table 1. There are no perilipin negative (dead) adipocytes in WT and *Lgals3*^{-/-} mice. Black arrows = macrophages.



Supplementary Figure 4. Unedited gel images of Western blot analysis of phospho-AKT (p-AKT, 60kDa) and total AKT (AKT, 60 kDa) in protein extracts from WT and *Lgals3*^{-/-} VAT cultures stimulated with insulin. Normalization for total protein content was performed on the same Stain-Free gel (lower box) using Stain-Free technology (Bio-Rad laboratories).