**Lyplal1** is dispensable for normal fat deposition in mice

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Summary Statement

We demonstrate that the Lyplal1 gene is dispensable in mice, with important implications for interpretation of GWAS results linking Lyplal1 to metabolism and fat distribution.

Abstract

Genome-wide association studies (GWAS) have detected association between variants in or near the Lysophospholipase-like 1 (LYPLAL1) locus and metabolic traits, including central obesity, fatty liver and waist-hip ratio. LYPLAL1 is also known to be upregulated in the adipose tissue of obese patients. However, the physiological role of LYPLAL1 is not understood. To investigate the function of Lyplal1 in vivo we investigated the phenotype of the Lyplal1tm1a(KOMP)Wtsi homozygous mouse. Body composition was unaltered in Lyplal1 knockout mice as assessed by Dual-energy X-ray absorptiometry (DEXA) scanning, both on normal chow and on a high fat diet. Adipose tissue distribution between visceral and subcutaneous fat depots was unaltered, with no change in adipocyte cell size. Response to both insulin and glucose dosing was normal in Lyplal1tm1a(KOMP)Wtsi homozygous mice, with normal fasting blood glucose concentrations. RNAseq analysis of liver, muscle and adipose tissue confirmed that Lyplal1 expression was ablated with minimal additional changes in gene expression. These results suggest that Lyplal1 is dispensable for normal mouse metabolic physiology and that despite having been maintained through evolution Lyplal1 is not an essential gene, suggesting possible functional redundancy. Further studies will be required to clarify its physiological role.

Introduction

Lysophospholipase-like 1 (LYPLAL1) is a protein with poorly understood biological role, despite its evolutionary conservation (Fig. S1). LYPLAL1 crystal structure is similar to APT1 (acyl protein thioesterase 1, also known as LYPLA1), but its active site shape indicates that unlike APT1, which depalmitoylates Gα and Ras proteins, it cannot bind long-chain substrates. Biochemical data confirms this, also demonstrating that LYPLAL1 accepts short-chain 4-nitrophenyl esters (Burger et al., 2012). Despite identification of a small molecule inhibitor, the natural substrate of LYPLAL1 and its physiological role remain unknown.

Genome-wide association studies (GWAS) have identified variants close to LYPLAL1 associated with various metabolic-related phenotypes including waist-hip ratio, with a greater
effect in females (Heid et al., 2010; Lindgren et al., 2009; Randall et al., 2013), subcutaneous/visceral white adipose tissue (scWAT/vWAT) ratio (Chu et al., 2017; Fox et al., 2012), BMI (Benjamin et al., 2011), fasting insulin levels, insulin resistance (Bille et al., 2011; Manning et al., 2012; Scott et al., 2012), insulin clearance (Goodarzi et al., 2013), increased fasting serum triglyceride levels in males (Bille et al., 2011) and nonalcoholic fatty liver disease (Speliotes et al., 2011). In addition the rs8486567 SNP downstream of \textit{LYPLAL1} was included in a genetic risk score associated with excess BMI loss after Roux-en Y gastric bypass surgery (Bandstein et al., 2016).

\textit{Lyplal1} is broadly expressed in mouse, with higher expression in WAT, liver, skeletal muscle and kidney. \textit{Lyplal1} expression was downregulated in a depot-specific manner in mice on a high fat diet (HFD) (Lei et al., 2015). Similarly, \textit{Lyplal1} expression was lower in kidney fat of Zucker diabetic fatty rats, compared to Zucker lean rats (Schmid et al., 2012), yet \textit{LYPLAL1} expression is increased in WAT from obese patients (Steinberg et al., 2007), and liver in mouse metabolic disease models (Ahn et al., 2016).

Selective inhibition of \textit{LYPLAL1} in cultured hepatocytes caused an increase in glucose production (Ahn et al., 2016), while adipocyte studies indicate that \textit{Lypalpha} is not required for adipocyte differentiation (Lei et al., 2015).

Given previous human GWAS results, and the pathophysiological alteration in gene expression levels, we hypothesised that \textit{LYPLAL1} is important for metabolic regulation. We characterised a \textit{Lyplal1} knockout mouse model, aiming to elucidate the function of \textit{Lyplal1 in vivo} and to establish whether \textit{LYPLAL1} is a plausible causal gene at this locus.

\textbf{Results}

\textbf{Verification of Lyplal1 knockout}

Mice with loss of \textit{Lyplal1}, (termed \textit{Lyplal1}^{tm1a/tm1a}) were generated using the tm1a knockout first allele design as part of the IMPC project (Fig. 1A). \textit{Lyplal1} mRNA levels were negligible in all organs tested, with the tm1a allele resulting in >95% knockout of \textit{Lyplal1} at the RNA level in the kidney and gastrocnemius muscle, and >99% knockout in all other tissues tested (heart, liver, spleen, adipose, Fig. 1B-I). Furthermore, RNASeq confirmed loss of \textit{Lyplal1} expression, consistent with the gene construct, with only a few detectable reads mapping to exon 1, (Fig. S2). No other exons display complete coverage in any of the
Lyplal1<sub>tm1a/tm1a</sub> samples investigated, with no more than three reads at any one base outside exon 1. The reduction in reads for exon 1 may be due to disruption of an unmapped regulatory element such as an enhancer in the first exon or due to nonsense mediated decay. The allele design is such that transcription is prevented beyond the lacZ in the inserted cassette. However, in the case of any skipped splicing over the cassette, the resulting transcripts would be frameshifted and subject to nonsense mediated decay. It may also be possible that the Exon1:<LacZ transcript is detected as aberrant and degraded. Lyplal1 was undetectable in all protein samples collected from homozygous Lyplal1<sup>tm1a/tm1a</sup> mice (Fig. 1J, Fig. S3).

**Body Composition**

No obvious body weight or metabolic phenotype was observed for Lyplal1<sup>tm1a/tm1a</sup> mice during standardized phenotyping (http://www.mousephenotype.org/data/genes/MGI:2385115) (White et al., 2013). To further investigate the role of Lyplal1 on adipose tissue development, and other relevant metabolic phenotypes, mice were challenged with a HFD from 6 weeks of age. Body weights, and nose to tailbase length, were not altered in HFD-fed Lyplal1<sup>tm1a/tm1a</sup> mice, compared to wild-type (Fig. 2A and Fig. S4A-B). Lean and fat mass composition, as well as bone mineral parameters, were also unaltered in Lyplal1<sup>tm1a/tm1a</sup> mice at 14 and 24 weeks of age (Fig. 2B-D, S4C-K). To investigate fat distribution, vWAT, scWAT and brown adipose tissue was dissected from 28-week-old mice and weighed. No change was detected in Lyplal1<sup>tm1a/tm1a</sup> mice (Fig. 2E-G), indicating that Lyplal1 is dispensable for adipose tissue distribution and size in mice. All other organ weights measured (liver, kidney, gastrocnemius and tibialis anterior muscles, heart, spleen) were similar to wildtype in Lyplal1<sup>tm1a/tm1a</sup> mice at 28 weeks of age (Table S1).

To determine whether adipose tissue architecture was altered, adipocyte CSA was determined from scWAT sections (Fig. 2H-I). No changes were observed, either in average adipocyte CSA (Fig. 2I) or in distribution of cell CSA (Supplementary Fig S5). Furthermore, liver cryosections showed no qualitative differences in Lyplal1<sup>tm1a/tm1a</sup> mice compared to wild-type (Fig. 2J), indicating that knockout of Lyplal1 does not alter adipocyte size, nor the extent of fatty liver in adult mice on a HFD.
Plasma lipid concentration (Cholesterol, HDL, LDL, NEFAC, Triglycerides), as well as other metabolically relevant parameters (Albumin, ALP, ALT, Amylase, AST, Creatine Kinase, Creatinine, Fructosamine, Glycerol) were determined in 28-week-old mice after a 4 h fast (Table 1). No parameters were altered in Lyplal1tm1a/tm1a mice compared to wild-type, again suggesting that Lyplal1 does not influence the regulation of these parameters.

**Glucose homeostasis**

Due to potential links with fasting insulin levels, insulin clearance and insulin resistance, we investigated glucose homeostasis in Lyplal1tm1a/tm1a mice. Fasting blood glucose was measured at three ages after varying lengths of fast. Although levels differed due to sex, Lyplal1 knockout did not alter fasting blood glucose in any conditions tested (Fig. S6). The responses to insulin and glucose doses were tested via intraperitoneal insulin and glucose tolerance tests at 18 and 22 weeks of age respectively. Lyplat1tm1a/tm1a mice did not show an altered response to either challenge (Fig. 3A-D). Plasma insulin levels in 28-week-old Lyplat1tm1a/tm1a mice after a 4 h fast were also unaltered (Fig. 3E). Collectively, these results indicate that loss of Lyplal1 does not profoundly alter glucose homeostasis.

**Indirect Calorimetry**

Indirect calorimetry was performed on 26-week-old mice for 48 h, to collect data on food intake, energy expenditure and activity levels. Activity levels and food intake during calorimetry were normal in Lyplal1tm1a/tm1a animals (Fig. 3F, S7A,B,G,H). Whilst there was a small significant difference in VO2 in Lyplal1tm1a/tm1a mice, energy expenditure, VCO2 and RER remained unaltered, and traces through the time period were all qualitatively similar (Fig. 3G-H, S7C-F,J). Overall, the calorimetry did not demonstrate major changes in energy homeostasis due to Lyplal1 loss.

**RNAseq**

As no phenotype alterations were observed in Lyplal1tm1a/tm1a mice, we sought to investigate whether there were compensatory changes in gene expression that might explain the apparent redundancy in Lyplal1 function. We performed differential gene expression analysis of RNAseq data obtained from metabolically relevant tissues (liver, skeletal muscle (gastrocnemius), scWAT and vWAT). As expected, Lyplal1 was the top differentially expressed gene between knockout and wild-type for all tissues, with additional differentially expressed genes (p<0.05, adjusted for multiple testing) listed in Table 2. Lyplal1 was the only...
differentially regulated gene in gastrocnemius muscle, with only one additional gene differentially regulated in liver (Nuak1), three additional genes in scWAT and 11 additional genes in vWAT. Despite meeting the significance threshold, all these additional genes have low log-fold-changes (0.2-0.75), indicating that knockout of Lyplal1 does not cause substantial changes in the transcriptome in mice.

**Discussion**

*LYPLAL1* has been linked to many metabolic phenotypes in humans and rodents, through GWAS and expression studies (Bandstein et al., 2016; Benjamin et al., 2011; Bille et al., 2011; Chu et al., 2017; Fox et al., 2012; Goodarzi et al., 2013; Heid et al., 2010; Lei et al., 2015; Lindgren et al., 2009; Manning et al., 2012; Randall et al., 2013; Schmid et al., 2012; Scott et al., 2012; Speliotes et al., 2011; Steinberg et al., 2007). However, these results are correlative and do not demonstrate direct causality of *LYPLAL1* on associated phenotypes. We therefore aimed to investigate the *in vivo* role of *Lyplal1* in metabolic regulation, and adipose tissue deposition. We studied *Lyplal1<sup>tm1a/tm1a</sup>* mice obtained from the KOMP repository, with primary phenotyping obtained by MGP (White et al., 2013).

In our hands, *Lyplal1<sup>tm1a/tm1a</sup>* mice did not display a detectable metabolic phenotype, even under a HFD challenge. *Lyplall* expression loss was confirmed at both the RNA and protein level, with RNASeq data showing few detectable reads mapping to exon 1, consistent with the gene construct. This suggests that the lack of obvious phenotype was not due to residual *Lyplall* expression. In the absence of a detectable phenotype, there was the possibility that another gene was compensating *Lyplall*. However, our RNAseq data demonstrates minimal expression changes in *Lyplal1<sup>tm1a/tm1a</sup>* mice, with small alterations in genes unrelated to metabolism, suggesting that *Lyplall* is dispensable for normal fat deposition and metabolic control in mice, despite its evolutionary conservation.

Whilst the evidence implicating the *LYPLAL1* locus in regulation of fat distribution and metabolism was compelling, it is important to remember that the SNPs identified by GWAS are not within *LYPLAL1* itself, and the underlying causal gene or effector transcript has yet to be identified. It is therefore highly plausible that these phenotypes are regulated via other genes. This has also been well documented in the case of the identification of the causal gene around the *FTO* locus (Church et al., 2009; Claussnitzer et al., 2015; McMurray et al., 2013; Smemo et al., 2014; Stratigopoulos et al., 2014), and reinforces the caution that must be taken...
when interpreting GWAS results. Further studies, focused on fine-mapping the \textit{LYPLAL1} locus, and establishing a link between associated genetic variants and effects on expression and regulation of neighbouring genes are therefore warranted to elucidate the mechanisms through which these SNPs are acting. Indeed, similar to most other GWAS loci, this variant maps to a non-coding region which may be involved in modulating gene expression rather than eliminating gene function. Therefore, modulating expression of \textit{LYPLAL1}, and other nearby genes, in different cells, tissues and developmental stages may be required to further inform the likely effector transcript at this locus, and fully test whether \textit{Lyplal1} changes in expression may contribute to the phenotype.

In conclusion, \textit{Lyplal1} is dispensable for normal adipose and metabolic regulation in these mice for all parameters and conditions tested here. Whilst there are differences between mice and humans, and between mouse strains, this has important implications for the interpretation of GWAS results linking SNPs close to \textit{LYPLAL1} to metabolic phenotypes and highlights the challenges in establishing causality based on GWAS results.

\textbf{Materials and Methods}

\textbf{Animal Studies}

All experiments were carried out in accordance with UK Home Office regulations, UK Animals (Scientific Procedures) Act 1986 and with approval from Wellcome Trust Sanger Institute’s Animal Welfare Committee. All mice were maintained in specific pathogen free facilities in individually ventilated cages at standard temperature (19-23$^\circ$C) and humidity (55% ±10%), on a 12h dark, 12h light (0730-1930) cycle. Sperm from C57BL6/N mice carrying the targeted, non-conditional allele \textit{Lyplal1}^{tm1a(KOMP)Wtsi} were obtained from the KOMP repository (http://www.komp.org/pdf.php?projectID=23342, see allele design in Fig. 1A) (Skarnes et al., 2011). Following confirmation of germ-line transmission, mice derived from heterozygous intercrosses were genotyped for the \textit{Lyplal1}^{tm1a} allele by PCR carried out as previously described (Ryder et al., 2013).

Mice underwent standardized phenotyping using a modified version of the Sanger Mouse Genetics Project pipeline detailed previously (White et al., 2013), using breeder’s chow (LabDiets 5021, 21% kcal as fat, LabDiet, London, UK) instead of a HFD. \textit{Lyplal1} data from the MGP pipeline and others is available online: http://www.mousephenotype.org/data/genes/MGI:2385115.
HFD studies using Research diets D12451 (45% kcal as fat, Research Diets, New Brunswick, NJ, USA) were performed in three batches, with 11 males and 10 females of each genotype switched from breeder’s chow to HFD at 6 weeks of age. Genotypes and sexes were mixed throughout the batches.

Body composition was measured under anaesthesia with ketamine (Ketaset®, Fort Dodge Animal Health, Overland Park, KS, USA) and xylazine (Rompun®, Bayer Animal Health, Leverkusen, Germany) using a PIXImus densitometer (GE Lunar, Madison, WI, USA). Nose to tailbase length was measured using a ruler with 1mm graduations prior to Dual-energy X-ray absorptiometry (DEXA). This was performed at 12 (100 mg kg\(^{-1}\) ketamine and 10 mg/kg xylazine) and 22 (90 mg kg\(^{-1}\) ketamine and 9 mg/kg xylazine) weeks of age. Quality control was performed using a calibrated phantom before imaging. Anaesthesia was reversed by ip injection of Atipamezole (1 mg kg\(^{-1}\) Antisedan, Orion Pharma, Espoo, Finland).

An intraperitoneal insulin tolerance test was performed on 16-week-old mice after a 6 h fast (0800-1400), using 0.6 U kg\(^{-1}\) Actrapid insulin (Novo Nordisk, Bagsvaerd, Denmark). An intraperitoneal glucose tolerance test was performed on 20-week-old mice after an overnight fast (from 1700, typically 16 h duration), using 2 g kg\(^{-1}\) glucose. Mice were individually house and approximately 0.5mm of the tail tip was removed with a scalpel blade and a fasting blood sample directly taken (Accu-chek Aviva, Roche, Indianapolis, IN, USA). After intraperitoneal injection, further blood samples were taken at 15, 30, 60 and 120 min post-injection. Area under the curve was calculated using GraphPad Prism.

Mice were individually housed for 48 h of indirect calorimetry cages (LabMaster system, TSE-systems, Bad Homburg, Germany) at 24 weeks of age. A final blood glucose reading was collected from 26-week-old mice after a 4 h fast, followed by anaesthesia (100 mg kg\(^{-1}\) ketamine and 10 mg kg\(^{-1}\) xylazine) and culling, with retro-orbital blood and multiple tissue samples collected.
**Ex vivo analysis**

Heparinised whole-blood samples were centrifuged at 5,000 rcf for 10 min at 4°C, and the separated plasma analysed using an Olympus AU400 (Olympus, Tokyo, Japan). Insulin concentrations were determined by ELISA, according to the manufacturer’s instructions (Millipore, Billerica, MA, USA).

Protein extracts were generated from frozen tissue samples by homogenization in TPER buffer supplemented with Halt protease and phosphatase inhibitor cocktail (both Thermo Scientific, Rockfold, IL, USA), using an Omni TH Tissue homogenizer (Omni International, NW Kennesaw, GA, USA). Western blotting of tissue protein extracts was performed using standard protocols, using the blocking solutions (in TBS with 0.1% Tween 20) and antibodies listed below:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source Company</th>
<th>Dilution</th>
<th>Blocking solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit anti-Lypal1</td>
<td>Proteintech Group, Rosemont, IL, USA</td>
<td>1:1000</td>
<td>3% [w/v] milk</td>
</tr>
<tr>
<td>Mouse anti-GAPDH</td>
<td>Abcam, Cambridge, UK</td>
<td>1:5000</td>
<td>3% [w/v] BSA</td>
</tr>
<tr>
<td>Goat anti-rabbit</td>
<td>Bio-Rad, Hercules, CA, USA</td>
<td>1:10000</td>
<td>3% [w/v] milk</td>
</tr>
<tr>
<td>Goat anti-mouse</td>
<td>Bio-Rad, as above</td>
<td>1:10000</td>
<td>3% [w/v] BSA</td>
</tr>
</tbody>
</table>

The blots were visualised using Amersham ECL reagents (GE Healthcare, Chicago, IL, USA) and developed using a Xograph Imaging Systems Compact X4.

5 µm scWAT paraffin sections were stained with Haematoxylin and Eosin (H&E) using a Leica ST5020 Multistainer machine and a Leica CV5030 Cover Slipper (Leica, Wetzlar, Germany). 10 µm liver cryosections were stained with H&E or OilRedO and haematoxylin by conventional methods. Images were collected using a Leica stereomicroscope and a Hamamatsu slide scanner (Hamamatsu, Japan). Adipocyte cross-sectional area (CSA) analysis was calculated from one section of 1.8 x 1.34 mm per mouse using ImageJ software (NIH).

**RNA extraction and qPCR**

RNA was extracted from snap frozen mouse tissue using the Qiagen RNeasy plus Universal kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). RNA concentration and purity was evaluated by NanoDrop (Thermo Scientific, Wilmington, DE,
USA). RNA integrity was further assessed using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). cDNA synthesis was performed using 500 ng – 1 µg RNA, random primers and Superscript II reverse transcriptase (Life Technologies, Carlsbad, CA, USA). qPCR was performed using Sybr Green (Applied Biosystems, Foster City, CA, USA) and run on an AB7500 qPCR machine (Applied Biosystems). The primers used were as follows:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S</td>
<td>GTAACCCGTTGAACCCATT</td>
<td>CCATCCCAATCGGTAGTAGCG</td>
</tr>
<tr>
<td>Gapdh</td>
<td>TGGTTACACCCCATCACAACA</td>
<td>GGTGAAGGTCGGTGAACGG</td>
</tr>
<tr>
<td>Lyplal1</td>
<td>CACGGCTCAGGTCACTCTTG</td>
<td>AGGGGCGTTGGATAATG</td>
</tr>
<tr>
<td>Rpl32</td>
<td>GGCCAGATCTCTGATGCCCAAC</td>
<td>CAGCTGTGCTGCTCTTCTAC</td>
</tr>
</tbody>
</table>

Relative expression for Lyplal1 was calculated using the $\Delta\Delta C_t$ method, relative to the cubic mean of three reference genes (Livak and Schmittgen, 2001).

RNAseq

RNAseq was performed on five samples per sex and genotype from gastrocnemius, liver, scWAT and vWAT. Libraries were prepared using the Illumina TruSeq Stranded mRNA Library Preparation Kit with 10 PCR cycles (Illumina, San Diego, CA, USA) Pools of 20 samples (one pool per organ) were run on three lanes each with 75 bp paired end runs on an Illumina HiSeq 2000 with v4 chemistry, with each pool run on three lanes. Reads were aligned to the NCBI m38 version of the mouse genome, data from multiple lanes combined using Samtools v1.3, mapped reads counted using Feature Counts with duplicates retained and differential expression analysed using DESeq2 (Love et al., 2014). Outliers which did not cluster with the appropriate tissue on the PCA plot were removed. The data was visualised using IGV to confirm the location of any remaining reads in knockout samples (Robinson et al., 2011). RNAseq data is available through the European Nucleotide Archive (ENA), study number PRJEB14194.
**Statistical analysis**

Unless otherwise stated, statistical analysis was performed as described in Karp et al (Karp et al., 2012), using PhenStat version 2.3.2, using the mixed model framework (Kurbatova et al., 2015). Multiple testing was managed by controlling the family wise error rate to 5% using the Holm method (Holm, 1979).

\[ Y \sim \text{Diet} + \text{Sex} + \text{Diet*Sex} + (1|\text{Batch}) \]  

[Eq. 1]

For indirect calorimetry, data analysis was performed in R, using linear model analysis and correcting for body weight when assessing energy expenditure, VO$_2$ & VCO$_2$, and change in body weight when assessing food intake and RER. Interaction terms were checked and were not significant, therefore these were excluded from the model.

\[ \text{Model} \leftarrow \text{lm}(\text{Variable} \sim 1 + \text{Genotype} + \text{Sex} + \text{Weight} + \text{Batch}) \]  

[Eq. 2]

**Acknowledgements**

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**Abbreviations**

CSA – cross sectional area  
DEXA – dual-energy x-ray absorptiometry  
GWAS – genome-wide association study  
H&E – haematoxylin and Eosin  
ipGTT – intraperitoneal glucose tolerance test  
ipITT – intraperitoneal insulin tolerance test
RER – respiratory exchange ratio
scWAT – subcutaneous white adipose tissue
TPER – tissue protein extraction reagent
vWAT – visceral white adipose tissue
WAT – white adipose tissue

Author Contributions
R.A.W. conceived, designed and performed experiments, analysed and interpreted the data and wrote the manuscript. A.S.G. performed experiments, analysed the data and approved the final manuscript. E.H.W. and F.E.C. performed experiments and approved the final manuscript. A.G. conceived and designed experiments and critically reviewed the manuscript. C.J.L. conceived, designed and performed experiments, interpreted the data, critically reviewed the manuscript and supervised the research activity. I.B. conceived and designed experiments, interpreted the data, edited and critically reviewed the manuscript and supervised the research activity. All authors critically reviewed and approved the final version of the manuscript.

Competing Interests
No competing interests declared.

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References


Table 1 – Concentrations of the listed parameters in plasma collected from 28-week-old knockout mice. Blood was collected retroorbitally after a 4 h fast and plasma analysed using an Olympus AU400. ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; CK = creatine kinase; HDL = high density lipoprotein; LDL = low density lipoprotein; NEFAC = non-esterified fatty acids (n=9 male Lyplal1<sup>+/+</sup>, n=9 male Lyplal1<sup>tm1a/tm1a</sup>, n=9 female Lyplal1<sup>+/+</sup>, n=7 female Lyplal1<sup>tm1a/tm1a</sup>).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male Lyplal1&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>Male Lyplal1&lt;sup&gt;tm1a/tm1a&lt;/sup&gt;</th>
<th>Female Lyplal1&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>Female Lyplal1&lt;sup&gt;tm1a/tm1a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>27.07 ± 1.575</td>
<td>26.89 ± 1.508</td>
<td>28.26 ± 0.974</td>
<td>28.26 ± 0.974</td>
</tr>
<tr>
<td>ALP (U l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>81.60 ± 16.61</td>
<td>88.79 ± 22.67</td>
<td>96.50 ± 19.31</td>
<td>99.78 ± 14.95</td>
</tr>
<tr>
<td>ALT (U l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>213.9 ± 103.7</td>
<td>223.6 ± 110.6</td>
<td>111.9 ± 45.9</td>
<td>115.5 ± 43.57</td>
</tr>
<tr>
<td>Amylase (U l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>571.8 ± 44.44</td>
<td>577.4 ± 62.48</td>
<td>505.1 ± 43.09</td>
<td>519.4 ± 45.16</td>
</tr>
<tr>
<td>AST (U l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>218.9 ± 67.96</td>
<td>202.1 ± 88.21</td>
<td>171.8 ± 27.64</td>
<td>164.7 ± 50.08</td>
</tr>
<tr>
<td>Cholesterol (mmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>5.411 ± 0.861</td>
<td>5.404 ± 0.823</td>
<td>3.932 ± 0.516</td>
<td>3.816 ± 0.520</td>
</tr>
<tr>
<td>CK (U l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>359.1 ± 162.8</td>
<td>290.1 ± 194.0</td>
<td>361.6 ± 228.7</td>
<td>252.0 ± 234.7</td>
</tr>
<tr>
<td>Creatinine (µmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>8.911 ± 1.040</td>
<td>7.878 ± 1.353</td>
<td>9.578 ± 0.821</td>
<td>9.629 ± 1.805</td>
</tr>
<tr>
<td>Fructosamine (µmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>193.9 ± 8.385</td>
<td>189.3 ± 7.424</td>
<td>197.3 ± 6.907</td>
<td>196.9 ± 9.395</td>
</tr>
<tr>
<td>Glycerol (µmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>199.4 ± 28.32</td>
<td>204.7 ± 32.66</td>
<td>277.5 ± 58.06</td>
<td>293.2 ± 75.34</td>
</tr>
<tr>
<td>HDL (mmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.449 ± 0.392</td>
<td>3.416 ± 0.396</td>
<td>2.662 ± 0.241</td>
<td>2.583 ± 0.336</td>
</tr>
<tr>
<td>LDL (mmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.122 ± 0.288</td>
<td>1.130 ± 0.224</td>
<td>0.732 ± 0.106</td>
<td>0.695 ± 0.099</td>
</tr>
<tr>
<td>NEFAC (mmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.502 ± 0.080</td>
<td>0.576 ± 0.121</td>
<td>0.570 ± 0.103</td>
<td>0.684 ± 0.190</td>
</tr>
<tr>
<td>Triglycerides (mmol l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.444 ± 0.065</td>
<td>0.476 ± 0.076</td>
<td>0.458 ± 0.067</td>
<td>0.493 ± 0.118</td>
</tr>
</tbody>
</table>
Table 2 – Table listing the differentially regulated genes identified by DESeq2 analysis of RNAseq data (lfc = log2 fold change, differentially expressed genes filtered using padj < 0.05).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Gene Name</th>
<th>Ensemble ID</th>
<th>lfc</th>
<th>pvalue</th>
<th>padj</th>
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<tbody>
<tr>
<td></td>
<td>Lyplal1</td>
<td>ENSMUSG00000039246</td>
<td>-0.6533</td>
<td>1.71E-33</td>
<td>5.65E-29</td>
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<tr>
<td>Liver</td>
<td>Lyplal1</td>
<td>ENSMUSG00000039246</td>
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<td>p&lt;1E-200</td>
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<td>Nuak1</td>
<td>ENSMUSG00000020032</td>
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<td>0.0491</td>
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<tr>
<td>scWAT</td>
<td>Lyplal1</td>
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<td>2.13E-150</td>
<td>7.68E-146</td>
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<tr>
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<td>Entpd1</td>
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<td>-0.3180</td>
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<tr>
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<td>S1pr2</td>
<td>ENSMUSG00000000</td>
<td>-0.4830</td>
<td>4.66E-06</td>
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<td>2.26E-05</td>
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</tbody>
</table>
Figure 1 – Mice were generated with the Lyplal1\textsuperscript{tm1a} allele, resulting in knock out at both the protein and RNA level.

A: Diagram showing the Lyplal1\textsuperscript{tm1a} allele design (figure obtained from IMPC, http://www.mousephenotype.org/data/genes/MGI:2385115#order2). RNA & Protein were extracted from organs from 28-week-old mice. C-J: qPCR analysis of Lyplal1 mRNA levels in gastrocnemius (B), heart (C), liver (D), kidney (E), spleen (F), BAT (G), scWAT (H) and vWAT (I). Data are presented as means ± SD. Black triangles = male Lyplal1\textsuperscript{+/+}, white triangles = male Lyplal1\textsuperscript{tm1a/tm1a}, black circles = female Lyplal1\textsuperscript{+/+}, white circles = female Lyplal1\textsuperscript{tm1a/tm1a}. J: Protein levels of Lyplal1 and GAPDH were determined by Western blot in liver, kidney, scWAT and vWAT lysates. Representative blots shown. (B: n=5 female Lyplal1\textsuperscript{tm1a/tm1a}, n=7 other groups; C.E-G: n=3 each group; D: n=7 Lyplal1\textsuperscript{+/+}, n=8 Lyplal1\textsuperscript{tm1a/tm1a}, H: n=8 male Lyplal1\textsuperscript{+/+}, n=6 male Lyplal1\textsuperscript{tm1a/tm1a}, n=5 female Lyplal1\textsuperscript{+/+}, n=5 female Lyplal1\textsuperscript{tm1a/tm1a}, I: n=5 male Lyplal1\textsuperscript{+/+}, n=3 male Lyplal1\textsuperscript{tm1a/tm1a}, n=5 female Lyplal1\textsuperscript{+/+}, n=6 female Lyplal1\textsuperscript{tm1a/tm1a}; J: n=3 samples from each sex and genotype per tissue)
Figure 2 – *Lyplal1* knockout does not alter body composition in *Lyplal1* knockout mice fed high fat diet from 6 weeks of age.

A: Body weights of mice up to 28 weeks of age were not altered by genotype. B-D: Lean mass (B), fat mass (C) and fat percentage (D) were unaltered in 24-week-old mice, measured by DEXA. E-G: Subcutaneous white adipose tissue (scWAT, E), visceral white adipose tissue (vWAT, F) and brown adipose tissue (BAT) mass were unchanged 28-week-old mice, weighed after dissection. H: No qualitative change in scWAT morphology in knockout mice. Representative images shown of scWAT sections from 28-week-old mice stained with haematoxylin and eosin (H&E). I: No change in adipocyte cross sectional area (CSA) determined using Image J analysis of scWAT sections stained with H&E, analysed using PhenStat. J: No change in fatty liver in knockout mice. Representative images shown of liver sections from 28-week-old mice stained with Oil Red O and eosin. Black triangles = male *Lyplal1*+/+, white triangles = male *Lyplal1*tm1atm1a, black circles = female *Lyplal1*+/+, white circles = female *Lyplal1*tm1atm1a. Data are presented as means ± s.d.. Mixed Model analysis.
performed using PhenStat. (B-D: n=10 male \textit{Lyplal1}^{+/+}, n=11 male \textit{Lyplal1}^{tm1a/tm1a}, n=9 female \textit{Lyplal1}^{+/+}, n=8 female \textit{Lyplal1}^{tm1a/tm1a}; E,F: n=9 male \textit{Lyplal1}^{+/+}, n=10 male \textit{Lyplal1}^{tm1a/tm1a}, n=9 female \textit{Lyplal1}^{+/+}, n=7 female \textit{Lyplal1}^{tm1a/tm1a}; G: n=10 male \textit{Lyplal1}^{+/+}, n=10 male \textit{Lyplal1}^{tm1a/tm1a}, n=9 female \textit{Lyplal1}^{+/+}, n=8 female \textit{Lyplal1}^{tm1a/tm1a}; H,I: n=9 male \textit{Lyplal1}^{+/+}, n=8 male \textit{Lyplal1}^{tm1a/tm1a}, n=7 female \textit{Lyplal1}^{+/+}, n=8 female \textit{Lyplal1}^{tm1a/tm1a})
Figure 3 – Lyplal1 knockout does not alter glucose metabolism in mice.

A-D: Intraperitoneal insulin tolerance test at 18 weeks of age (ipITT, A) & intraperitoneal glucose tolerance test at 22 weeks of age (ipGTT, B) demonstrated no alteration in glucose clearance in knockout mice. Area under the curve was calculated for both ipITT (C) and ipGTT (D). AUC mixed model analysis performed using Phenstat. E: Plasma insulin levels after a 4 h fast were unaltered in 28-week-old knockout mice, determined by ELISA, mixed model analysis performed using PhenStat. F-H: Indirect calorimetry of mice at 26 weeks of age. No differences were observed in average activity count (F), respiratory exchange ratio (RER, G), or energy expenditure (H). Black triangles = male Lyplal1+/+, white triangles = male Lyplal1tm1a/tm1a, black circles = female Lyplal1+/+, white circles = female Lyplal1tm1a/tm1a, dashed line = male Lyplal1+/+, dotted line = male Lyplal1tm1a/tm1a, dash – dot line = female Lyplal1+/+, dash – dot – dot line= female Lyplal1tm1a/tm1a; Data are presented as means ± s.d., with linear regression lines as appropriate. (A,C: n=6 male Lyplal1+/+, n=6 male Lyplal1tm1a/tm1a, n=6 female Lyplal1+/+, n=5 female Lyplal1tm1a/tm1a; B,D: n=9 male Lyplal1+/+, n=9 male Lyplal1tm1a/tm1a).
n=9 male Lyplal1tm1a/tm1a, n=10 female Lyplal1+/+, n=8 female Lyplal1tm1a/tm1a; E: n=10 male Lyplal1+/+, n=11 male Lyplal1tm1a/tm1a, n=9 female Lyplal1+/+, n=8 female Lyplal1tm1a/tm1a; F-G: n=9 male Lyplal1+/+, n=8 male Lyplal1tm1a/tm1a, n=6 female Lyplal1+/+, n=7 female Lyplal1tm1a/tm1a; H: n=9 male Lyplal1+/+, n=8 male Lyplal1tm1a/tm1a, n=6 female Lyplal1+/+, n=6 female Lyplal1tm1a/tm1a)
Figure S1 – Figure demonstrates the conservation of Lypla1 across many species. Taken from Ensembl (www.ensembl.org).
Figure S2 — RNAseq results demonstrate that there are only a few detectable reads in exon 1 of \textit{Lyplal1}. Representative vWAT samples shown, upper two panels show wildtype samples and lower two panels show \textit{Lyplal1}^{tm1a/tm1a} samples. Figure was generated using IGV.
Figure S3 – Lyplal1 is knocked out at the protein level. Shown are the original Western blots. Blue marks on the left hand side correspond to Bio-Rad Dual Color Precision Plus Protein markers (250, 150, 100, 75, 50, 37, 25, 20, 15 kDa). Red arrows indicate the band for Lyplal1/GAPDH.
Figure S4 – *Lypal1* knockout does not alter body composition in *Lypal1* knockout mice fed high fat diet from 6 weeks of age.

A & B: Nose to tail base length was unaltered in 14-week-old (A) and 24-week-old (B) knockout mice. C-H: Lean mass (C), fat mass (D) and fat percentage (E), bone mineral density (BMD, F), bone mineral content (BMC, G) and bone area (H) were unaltered in 14-week-old knockout mice, measured by DEXA. I-J: BMD (I), BMC (J) and bone area (K) were unaltered in 24-week-old knockout mice, measured by DEXA.

Black triangles = male *Lypal1*+/-, white triangles = male *Lypal1*tm1a/tm1a, black circles = female *Lypal1*+/+, white circles = female *Lypal1*tm1a/tm1a. Data are presented as means ± s.d. (n=11 males, 9 females per genotype A-H, n=10 males per genotype, n=9 female *Lypal1*+/-, n=8 female *Lypal1*tm1a/tm1a I-K).
Figure S5 – Lyplal1 knockout does not alter distribution of adipocyte CSA.
Relative adipocyte cross sectional area (CSA) was determined using ImageJ analysis of scWAT sections stained with H&E, and grouped into bins of 1000 µm². Light blue = male Lyplal1+/+; dark blue = male Lyplal1tm1a/tm1a; light purple = female Lyplal1+/+; dark purple = female Lyplal1tm1a/tm1a. Data are presented as means ± s.d. (n=9 male Lyplal1+/+, n=8 male Lyplal1tm1a/tm1a, n=7 female Lyplal1+/+, n=8 female Lyplal1tm1a/tm1a).
Figure S6 – Fasting blood glucose was unaltered in Lyplal1 knockout mice after different length fasts and at different ages.

a: 22-week-old mice were fasted for 16 h overnight before blood glucose measurement. b: 18-week-old mice were fasted for 6 h before blood glucose measurement. c: 28-week-old mice were fasted for 4 h before blood glucose measurement.

Black triangles = male Lyplal1+/+; white triangles = male Lyplal1tm1ab/m1ab, black circles = female Lyplal1+/+; white circles = female Lyplal1tm1ab/m1ab. Data are presented as means ± s.d., mixed model analysis performed using PhenStat (A: n=6 male Lyplal1+/+; n=6 male Lyplal1tm1ab/m1ab, n=6 female Lyplal1+/+; n=5 female Lyplal1tm1ab/m1ab; B: n=10 male Lyplal1+/+; n=11 male Lyplal1tm1ab/m1ab, n=10 female Lyplal1+/+; n=8 female Lyplal1tm1ab/m1ab; C: n=10 male Lyplal1+/+; n=10 male Lyplal1tm1ab/m1ab, n=9 female Lyplal1+/+; n=8 female Lyplal1tm1ab/m1ab).
Figure S7 – Lyplal1 knockout does not cause large changes during 48 h indirect calorimetry at 26 weeks of age. Shaded areas show the periods of dark (1930-0730).

A & B: Food intake was unaltered by genotype or sex. C & D: There was a significant effect of genotype on VO2 (p=0.038 for genotype, linear model). E & F: VCO2 was unaltered by genotype or sex. G-J: Raw data graphs for X activity, Y activity, RER and energy expenditure.

Black or light blue triangles = male Lyplal1+/−, white or dark blue triangles = male Lyplal1tm1a/bm1a, black or light purple circles = female Lyplal1+/−, white or dark purple circles = female Lyplal1tm1a/bm1a. Data are presented as means ± s.d. (A-B: n=9 male Lyplal1+/−, n=8 male Lyplal1tm1a/bm1a, n=6 female Lyplal1+/−, n=5 female Lyplal1tm1a/bm1a; C-F: n=9 male Lyplal1+/−, n=8 male Lyplal1tm1a/bm1a, n=6 female Lyplal1+/−, n=6 female Lyplal1tm1a/bm1a; G-I: n=9 male Lyplal1+/−, n=8 male Lyplal1tm1a/bm1a, n=6 female Lyplal1+/−, n=7 female Lyplal1tm1a/bm1a).