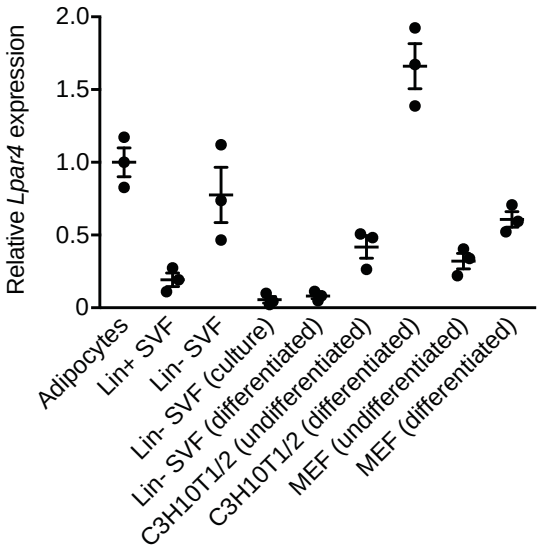
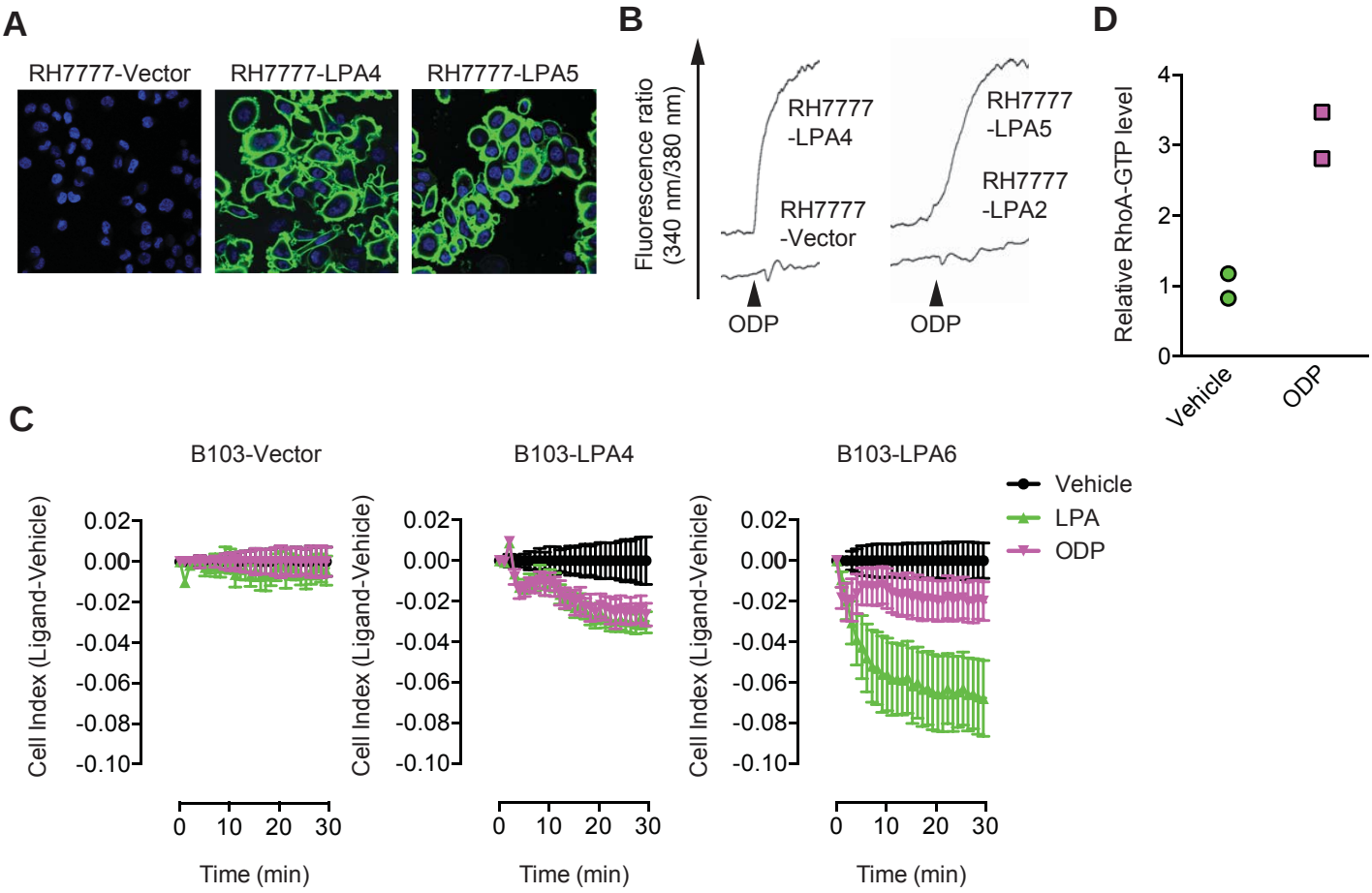
# Supplemental Figure 1



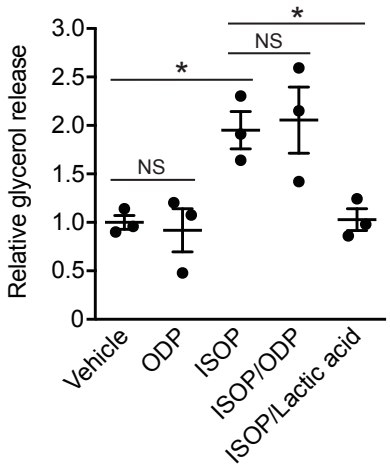

**Supplemental Figure 1. *Lpar4* expression in freshly isolated fractions of adipose tissue, cultured adipocyte progenitors, C3H10T1/2 cells and mouse embryonic fibroblasts (MEFs).**
Mouse *Lpar4* mRNA expression levels in adipocytes, lineage-positive (Lin+) and -negative (Lin-) populations of the stromal vascular fraction (SVF) isolated from WAT, along with cultured Lin- SVF containing adipocyte progenitors, C3H10T1/2 and MEFs are shown ($n$ = 3). Note that cultured Lin- SVF cells show substantially low expression of *Lpar4* even after adipocyte differentiation procedure. Each symbol represents the value from sample prepared from an independent experiment. Data are expressed as the mean ± SEM.

# Supplemental Figure 2




**Supplemental Figure 2. Octadecenyl phosphate (ODP), a full agonist for LPA4 and LPA5, activates RhoA.** **(A)** Confirmation of the heterologous expression of LPA4 or LPA5 in RH7777 cells. Representative anti-HA antibody (green) immunostaining images of RH7777 cells stably over-expressing HA-tagged LPA4 or LPA5 (RH7777-LPA4 or RH7777-LPA5, respectively) are shown. RH7777 cells transfected with empty vector (RH7777-Vector) were used as a negative control. The cells are counterstained with DAPI (blue). **(B)** Intracellular $Ca^{2+}$ responses to ODP (1 μM) in RH7777-LPA4 and RH7777-LPA5 cells. Representative traces of two independent experiments are shown. **(C)** ODP-induced impedance responses in B103 cells stably over-expressing LPA4 or LPA6 (B103-LPA4 or B103-LPA6, respectively). Real-time impedance traces after ODP (1 μM) or 1-oleoyl lysophosphatidic acid (LPA, 1 μM) were measured by the xCELLigence system. A unitless parameter called Cell Index (CI) representing impedance of electric flow was monitored, and CI after vehicle treatment is subtracted from that after ligand application to present the ligand-dependent impedance changes. B103 cells transfected with empty vector (B103-Vector) were used as a negative control. Data are expressed as the mean ± SEM (*n* = 4-6). **(D)** RhoA is activated by ODP. After the overnight serum-starvation, the C3H10T1/2-derived adipocytes were treated with vehicle or ODP (2.5 μM) for 1 min. The cell lysates were processed for the measurement of Rho-GTP levels with the colorimetric-based G-LISA activation assay kit. Each plot represents the mean values from technical duplicates. Results are representative of at least 2 independent experiments.

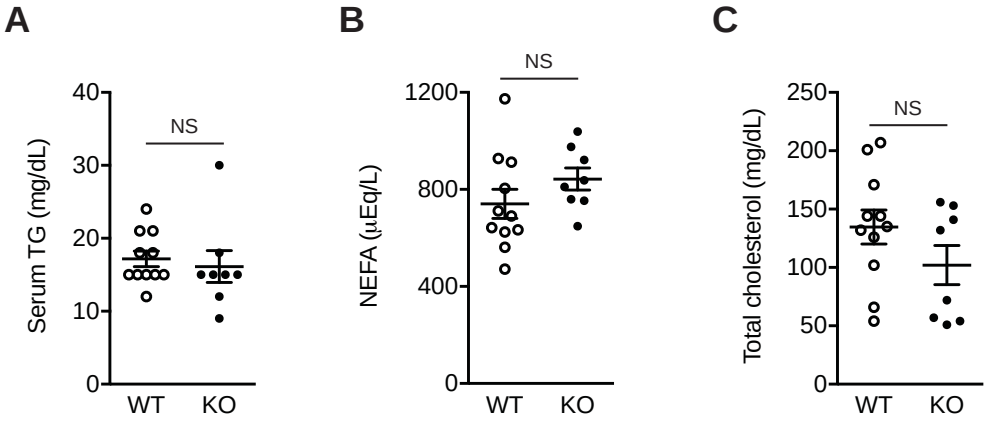
# Supplemental Figure 3




**Supplemental Figure 3. Octadecenyl phosphate (ODP) does not affect lipolysis in freshly isolated adipocytes.**

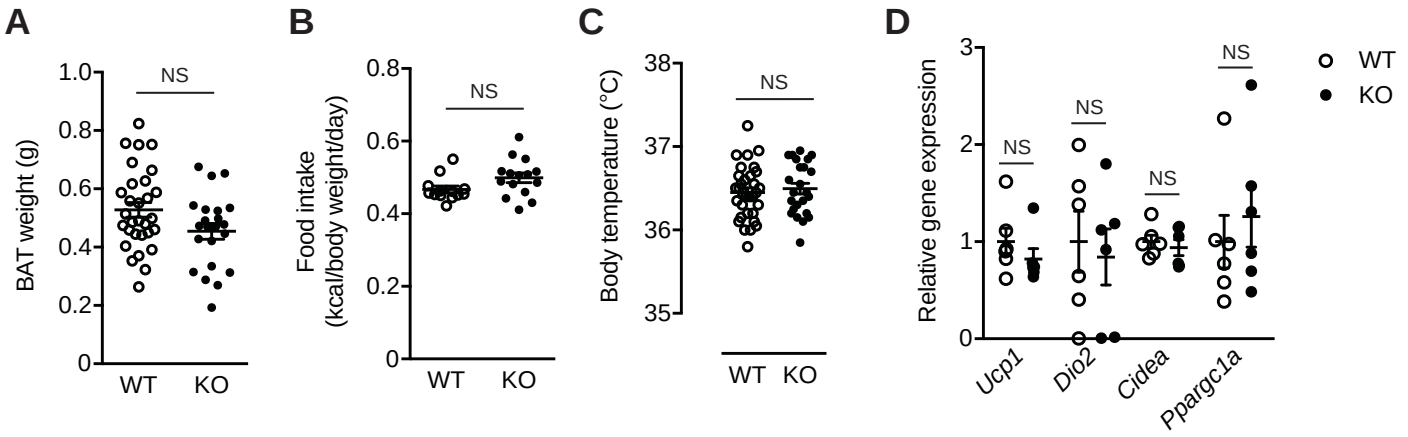
The primary adipocytes were collected from epididymal white adipose tissue (eWAT) of WT mice by enzymatic digestion and centrifugation, and stimulated with ODP (2.5 μM), isoproterenol (ISOP, 100 nM) and/or lactic acid (15 mM) for 2 h, and the glycerol concentration was determined by colorimetric detection. Data are expressed as the mean ± SEM ($n$ = 3). NS, not significant. *$P$ < 0.05, one-way ANOVA with Bonferroni's *post hoc* test. Representative data of two independent experiments are shown.

## Supplemental Figure 4



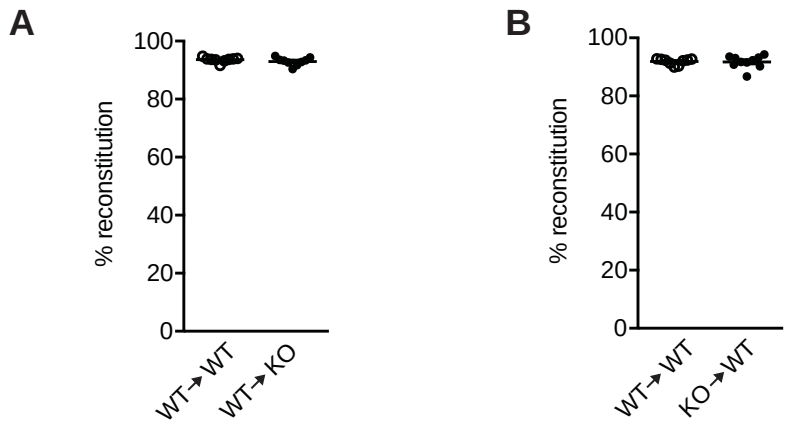

**Supplemental Figure 4. Serum lipid levels of WT and *Lpar4*-KO mice fed high-fat diet (HFD).**
Serum triglyceride (TG) **(A)**, non-esterified fatty acid (NEFA) **(B)**, and total cholesterol **(C)** levels of WT and *Lpar4*-KO mice fed HFD ($n$ = 8-13) are shown. Serum TG, NEFA, and cholesterol levels were determined by enzymatic reagents. Each symbol represents an individual mouse. Data are expressed as the mean ± SEM. NS, not significant, unpaired Student's *t*-test.

## Supplemental Figure 5



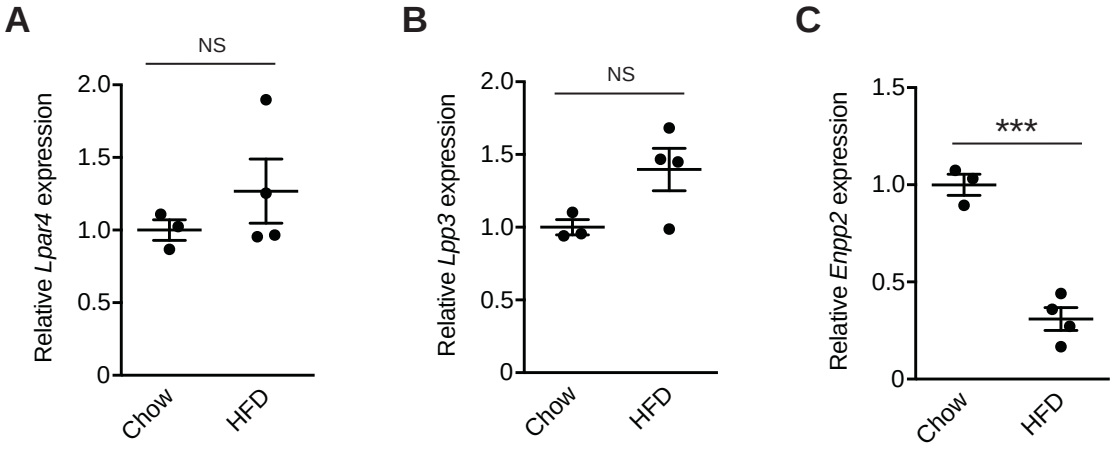

**Supplemental Figure 5. LPA4 ablation does not affect brown adipose tissue (BAT) weight, food intake, body temperature or BAT marker gene expression on high-fat diet (HFD).**
**(A-C)** BAT weight (**A**, *n* = 22-30), food intake (**B,** *n* = 13-15) and body temperature (**C,** *n* = 24-32) of WT and *Lpar4*-KO mice fed HFD. **(D)** Relative mRNA expression of BAT genes from HFD-fed WT and *Lpar4*-KO mice (*n* = 6 each). Each symbol represents an individual mouse. Data are expressed as the mean ± SEM. NS, not significant, unpaired Student's *t*-test.

## Supplemental Figure 6




**Supplemental Figure 6. The reconstitution efficiencies of bone marrow transfer experiments.** **(A)** Percentage of C57BL/6-Ly5.1-derived cells in peripheral blood of recipient WT or *Lpar4*-KO mice (*n* = 9 each). **(B)** Percentage of WT or *Lpar4*-KO mice-derived cells in peripheral blood of recipient C57BL/6-Ly5.1 mice (*n* = 9-10). Each symbol represents an individual mouse.

# Supplemental Figure 7




**Supplemental Figure 7. High-fat diet (HFD) feeding does not change mRNA expression of *Lpar4* or *Lpp3*, and rather decreases *Enpp2* expression in adipocytes.**
The mRNA expression for *Lpar4* (**A**), *Lpp3* (**B**), and *Enpp2* (**C**) in adipocytes was compared between chow-fed ($n$ = 3) and HFD-fed ($n$ = 4) mice by RT-qPCR analysis. eWAT was collected, enzymatically digested, and centrifuged to isolate adipocytes. Each symbol represents an individual mouse. Data are expressed as the mean ± SEM. NS, not significant. ***$P$ < 0.001, unpaired Student's $t$-test.

**Supplemental Table 1. Lists of significantly enriched GO terms found in up- or down-regulated genes in WAT of *Lpar4*-KO mice.**

Gene Ontology (GO) terms significantly enriched in 418 (eWAT) and 297 (iWAT) up-regulated, and 467 (eWAT) and 360 (iWAT) down-regulated genes in WAT from *Lpar4*-KO mice are shown. Gene annotation enrichment analysis of up-regulated probe sets was performed with Database for Annotation, Visualization and Integrated Discovery (DAVID). To extract the statistically overrepresented GO terms in differentially expressed genes (DEGs), we used modified Fisher's exact test with Benjamini and Hochberg false discovery rate (FDR) correction. A GO term with an FDR-corrected P-value < 0.05 was considered to be significantly enriched. GO terms significantly enriched in analyses of both eWAT and iWAT are shaded in gray.

## Up-regulated genes in WAT of *Lpar4*-KO mice

eWAT (418 genes)

Biological processes

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0006091 | generation of precursor metabolites and energy | 22 | 261 | 7.17E-04 |
| GO:0055114 | oxidation reduction | 37 | 672 | 8.52E-04 |
| GO:0060548 | negative regulation of cell death | 20 | 245 | 8.97E-04 |
| GO:0043066 | negative regulation of apoptosis | 20 | 239 | 1.03E-03 |
| GO:0043069 | negative regulation of programmed cell death | 20 | 244 | 1.05E-03 |
| GO:0006006 | glucose metabolic process | 13 | 140 | 1.13E-02 |
| GO:0015980 | energy derivation by oxidation of organic compounds | 11 | 98 | 1.16E-02 |
| GO:0019217 | regulation of fatty acid metabolic process | 6 | 19 | 1.22E-02 |
| GO:0010941 | regulation of cell death | 29 | 563 | 1.24E-02 |
| GO:0006732 | coenzyme metabolic process | 13 | 143 | 1.27E-02 |
| GO:0043067 | regulation of programmed cell death | 29 | 560 | 1.27E-02 |
| GO:0042981 | regulation of apoptosis | 29 | 553 | 1.36E-02 |
| GO:0010565 | regulation of cellular ketone metabolic process | 6 | 23 | 1.64E-02 |
| GO:0006916 | anti-apoptosis | 10 | 88 | 1.65E-02 |
| GO:0009628 | response to abiotic stimulus | 17 | 251 | 1.72E-02 |
| GO:0046486 | glycerolipid metabolic process | 12 | 129 | 1.73E-02 |
| GO:0006457 | protein folding | 12 | 127 | 1.74E-02 |
| GO:0009409 | response to cold | 5 | 14 | 2.01E-02 |
| GO:0051186 | cofactor metabolic process | 14 | 182 | 2.09E-02 |
| GO:0019216 | regulation of lipid metabolic process | 8 | 58 | 2.58E-02 |
| GO:0045333 | cellular respiration | 8 | 59 | 2.73E-02 |
| GO:0005996 | monosaccharide metabolic process | 14 | 191 | 2.88E-02 |
| GO:0019318 | hexose metabolic process | 13 | 169 | 3.15E-02 |
| GO:0006638 | neutral lipid metabolic process | 7 | 45 | 3.38E-02 |
| GO:0044093 | positive regulation of molecular function | 18 | 306 | 3.68E-02 |
| GO:0006084 | acetyl-CoA metabolic process | 6 | 31 | 3.82E-02 |
| GO:0019637 | organophosphate metabolic process | 13 | 176 | 3.85E-02 |
| GO:0008610 | lipid biosynthetic process | 17 | 285 | 4.82E-02 |
| GO:0009266 | response to temperature stimulus | 7 | 51 | 4.82E-02 |
| GO:0022900 | electron transport chain | 10 | 112 | 4.89E-02 |
| GO:0008015 | blood circulation | 10 | 111 | 4.89E-02 |
| GO:0003013 | circulatory system process | 10 | 111 | 4.89E-02 |

### Cellular components

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0005739 | mitochondrion | 88 | 1322 | 2.70E-1 |
| GO:0031090 | organelle membrane | 50 | 809 | 2.89E-0 |
| GO:0044429 | mitochondrial part | 39 | 524 | 3.83E-0 |
| GO:0031975 | envelope | 39 | 542 | 4.02E-0 |
| GO:0031967 | organelle envelope | 39 | 540 | 4.51E-0 |
| GO:0019866 | organelle inner membrane | 27 | 312 | 6.26E-0 |
| GO:0005743 | mitochondrial inner membrane | 26 | 296 | 7.95E-0 |
| GO:0031966 | mitochondrial membrane | 28 | 368 | 3.10E-0 |
| GO:0005740 | mitochondrial envelope | 29 | 391 | 3.37E-0 |
| GO:0031980 | mitochondrial lumen | 17 | 163 | 2.98E-0 |
| GO:0005759 | mitochondrial matrix | 17 | 163 | 2.98E-0 |
| GO:0000267 | cell fraction | 32 | 596 | 4.54E-0 |
| GO:0005625 | soluble fraction | 10 | 99 | 1.05E-0 |
| GO:0042175 | nuclear envelope-endoplasmic reticulum network | 12 | 160 | 2.33E-0 |
| GO:0005626 | insoluble fraction | 25 | 528 | 2.46E-0 |
| GO:0005624 | membrane fraction | 24 | 510 | 2.95E-0 |
| GO:0005792 | microsome | 12 | 176 | 4.32E-0 |
| GO:0042598 | vesicular fraction | 12 | 182 | 4.70E-0 |
| GO:0012505 | endomembrane system | 24 | 535 | 4.77E-0 |
| GO:0005783 | endoplasmic reticulum | 33 | 838 | 4.93E-0 |

### Molecular functions

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0048037 | cofactor binding | 22 | 226 | 8.89E-06 |
| GO:0050662 | coenzyme binding | 16 | 160 | 4.76E-04 |
| GO:0031406 | carboxylic acid binding | 10 | 85 | 1.15E-02 |
| GO:0000166 | nucleotide binding | 73 | 2183 | 1.19E-02 |

## iWAT (297 genes)

### Biological processes

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0055114 | oxidation reduction | 31 | 672 | 2.08E-05 |
| GO:0015980 | energy derivation by oxidation of organic compounds | 9 | 98 | 4.95E-02 |
| GO:0051050 | positive regulation of transport | 10 | 132 | 4.75E-02 |

### Cellular components

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0005739 | mitochondrion | 58 | 1322 | 9.89E-11 |
| GO:0044429 | mitochondrial part | 23 | 524 | 2.74E-03 |
| GO:0005759 | mitochondrial matrix | 12 | 163 | 3.79E-03 |
| GO:0031980 | mitochondrial lumen | 12 | 163 | 3.79E-03 |
| GO:0005625 | soluble fraction | 9 | 99 | 8.88E-03 |
| GO:0000267 | cell fraction | 23 | 596 | 7.31E-03 |

### Molecular functions

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0048037 | cofactor binding | 19 | 226 | 2.44E-06 |
| GO:0019842 | vitamin binding | 12 | 121 | 3.48E-04 |
| GO:0031406 | carboxylic acid binding | 10 | 85 | 6.02E-04 |
| GO:0030246 | carbohydrate binding | 17 | 317 | 1.90E-03 |
| GO:0050662 | coenzyme binding | 12 | 160 | 2.06E-03 |
| GO:0051287 | NAD or NADH binding | 6 | 44 | 3.22E-02 |

## Down-regulated genes in WAT of *Lpar4*-KO mice

### eWAT (467 genes)

#### Biological processes

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0007155 | cell adhesion | 33 | 561 | 4.66E-03 |
| GO:0022610 | biological adhesion | 33 | 562 | 2.42E-03 |
| GO:0001944 | vasculature development | 20 | 250 | 3.56E-03 |
| GO:0001568 | blood vessel development | 19 | 244 | 6.97E-03 |
| GO:0006954 | inflammatory response | 18 | 225 | 7.04E-03 |
| GO:0009611 | response to wounding | 22 | 347 | 1.80E-02 |
| GO:0007167 | enzyme linked receptor protein signaling pathway | 19 | 273 | 1.74E-02 |
| GO:0002526 | acute inflammatory response | 10 | 81 | 2.22E-02 |
| GO:0030198 | extracellular matrix organization | 11 | 101 | 2.20E-02 |
| GO:0048514 | blood vessel morphogenesis | 15 | 198 | 3.70E-02 |

#### Cellular components

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0005576 | extracellular region | 102 | 1680 | 1.49E-15 |
| GO:0031012 | extracellular matrix | 36 | 309 | 1.10E-11 |
| GO:0005578 | proteinaceous extracellular matrix | 35 | 297 | 1.25E-11 |
| GO:0044421 | extracellular region part | 58 | 774 | 1.21E-11 |
| GO:0044420 | extracellular matrix part | 12 | 92 | 1.08E-03 |
| GO:0005615 | extracellular space | 28 | 511 | 1.13E-02 |

#### Molecular functions

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0001871 | pattern binding | 33 | 561 | 1.04E-03 |
| GO:0030247 | polysaccharide binding | 33 | 562 | 1.04E-03 |
| GO:0019838 | growth factor binding | 20 | 250 | 1.76E-03 |
| GO:0005520 | insulin-like growth factor binding | 19 | 244 | 1.48E-03 |
| GO:0005539 | glycosaminoglycan binding | 18 | 225 | 2.04E-03 |
| GO:0030246 | carbohydrate binding | 22 | 347 | 2.34E-03 |
| GO:0003779 | actin binding | 19 | 273 | 1.52E-02 |
| GO:0042562 | hormone binding | 10 | 81 | 1.44E-02 |
| GO:0008201 | heparin binding | 11 | 101 | 4.58E-02 |
| GO:0008092 | cytoskeletal protein binding | 15 | 198 | 4.97E-02 |

### iWAT (360 genes)

#### Biological processes

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0042127 | regulation of cell proliferation | 23 | 538 | 4.19E-02 |
| GO:0022610 | biological adhesion | 24 | 562 | 4.51E-02 |
| GO:0048666 | neuron development | 16 | 292 | 4.53E-02 |
| GO:0043062 | extracellular structure organization | 11 | 149 | 4.68E-02 |
| GO:0042325 | regulation of phosphorylation | 16 | 290 | 4.81E-02 |
| GO:0044093 | positive regulation of molecular function | 16 | 306 | 4.97E-02 |

#### Cellular components

| GO ID | GO term | No. DEGs | No. listed genes | *P*-value |
|---|---|---|---|---|
| GO:0005576 | extracellular region | 64 | 1680 | 3.20E-06 |
| GO:0044421 | extracellular region part | 38 | 774 | 9.49E-06 |
| GO:0031012 | extracellular matrix | 19 | 309 | 1.38E-03 |
| GO:0005578 | proteinaceous extracellular matrix | 18 | 297 | 2.24E-03 |
| GO:0044420 | extracellular matrix part | 9 | 92 | 1.45E-02 |
| GO:0005615 | extracellular space | 22 | 511 | 2.06E-02 |

**Supplemental Table 2. Primers used for PCR analysis**

| Gene | Forward | Reverse |
|---|---|---|
| *Lpar1** | CGCCAGAGGACTATGAGGATGT | CAGCAGACAATAAAGGCACCAAG |
| *Lpar2*[#] | TCAGCCTAGTCAAGACGGTTGTC | CCAGAACGTTGCAGGACTTACAG |
| *Lpar2*[##] | CCGCTACCGAGAGACCACAC | ACTTACAGTCCAGGCCATCCA |
| *Lpar3*[#] | CCAACGTCTTATCTCCACACACC | CTTCACGTGTTGCACGTTACACT |
| *Lpar3*[##] | TGCTCATTCTGCTGGTGTGG | TGATGAAGAAGGCCAGGAGGT |
| *Lpar4** | GTCAACAATGCGACCACCAC | AAGCACCACAGAAGAACAAGAAACA |
| *Lpar5** | TCAGCCAACACGACTTCTACCA | GAGCGTTGAGAGGGAGACCA |
| *Lpar6** | GGTCATCTTCTGTTTCTGTTTTGTG | TGAGTTCTGAATTGTGTCTGAGGTG |
| *Ucp1* | ACACGGGGACCTACAATGCTTAC | CGTCATCTGCCAGTATTTTGTTG |
| *Dio2* | CAGCTTCCTCCTAGATGCCTACAA | CTGCACTGGCAAAGTCAAGAAG |
| *Cidea* | GCAACCAAAGAAATCGGGAATAG | CAGCATAGGACATAAACCTCAGCA |
| *Ppargc1a* | CAGAGAGAGAGGCAGAAGCAGAA | CAGGTGTAACGGTAGGTGATGAAAC |
| *Ppara* | GTGGCTGCTATAATTTGCTGTGG | AGCTTTGGGAAGAGGAAGGTGT |
| *Esrra* | GGCCACTCTCTGTGACCTTTTT | CAGCACTTCCATCCACACACTC |
| *Nrf1* | GCAACAGGGAAGAAACGGAAA | GGAGGGTGAGATGCAGAGTACAA |
| *Tfam* | AGCTGATGGGTATGGAGAAGGAG | GCTGAACGAGGTCTTTTTGGTTT |
| *Cox8b* | CATTCAGGGTGCCTCTTTGG | GGAGTTTTGGCTGGCTTGG |
| *Cyc1* | GCCGAAAGGTGATGCTGTC | TCCGAATGCTGGTGTGGTC |
| *Cycs* | CCAAATCTCCACGGTCTGTTC | TCTGCCCTTTCTCCCTTCTTC |
| *Idh3a* | ATCACCGAAGAAGCAAGCAAG | AGAAAGAGCCCATCTGACATCC |
| *Aco2* | AAGTGGGACGGCAAAGACC | ACCGATGAGCAGGTTGTTAGAGA |
| *Cd68* | CTCCCTGTGTGTCTGATCTTGCT | CTGTGCTTTCTGTGGCTGTAGGT |
| *Emr1* | CCACTCACCTGCTGCTACTCATT | CAGCACAGACCTCTCTCTTGAGC |
| *Tnfa* | CCAGACCCTCACACTCAGATCAT | TGGCACCACTAGTTGGTTGTCTT |
| *Ccl2* | CCACTCACCTGCTGCTACTCATT | CAGCACAGACCTCTCTCTTGAGC |
| *Col1a1* | GAACCCCAAGGAAAAGAAGCAC | GTGGACATTAGGCGCAGGAA |
| *Col6a3* | AGCACACCGAGCATCCAGTT | GTCCACACAAGTCCCAGCATC |
| *Fgf1a* | AAGCCTCCCAGAGCAGACAGA | CAGTTTGGGCTTTTTGTAGTTTCC |
| *Pparg2* | TATGCTGTTATGGGTGAAACTCTGG | GTCAAAGGAATGCGAGTGGTCT |
| *Cebpa* | CCCAGAGGACCAATGAAATGAAG | GTGTGTATGAACTGGCTGGAGGT |
| *Fasn* | TGGTCACAGATGATGACAGGAGA | GGGTTGATACCTCCATCCACAAT |
| *Lpl* | CAACAAGGTCAGAGCCAAGAGAA | TGCCGTACAGAGAAATCTCGAAG |
| *Srebp1c* | GGCACTGAAGCAAAGCTGAATAA | TCATGCCCTCCATAGACACATCT |
| *Adipoq* | AAAGGAGATGCAGGTCTTCTTGG | TGAACGCTGAGCGATACACATAA |
| *Cyp2f2* | ACCCAAACCTCTCCCAATCCT | TGAACACCGACCCATACTCCTT |

*Used for RT-PCR followed by gel electrophoresis (Figure 2A) and qRT-PCR analyses.
[#]Used only for RT-PCR followed by gel electrophoresis (Figure 2A).
[##]Used only for qRT-PCR analyses.