Supplementary Materials

Supplementary Table 1. List of primers used for quantitative PCR analysis.

Gene name	Gene symbol	Accession IDs	Sequence range	Product size (bp)	Primer sequences
β-actin	*Actb*	gi|145966868	851-1028	178	Forward 5'-AGT GTG ACG TTG ACA TCC GT-3'; Reverse 5'-CCA CCG ATC CAC ACA GAG TA-3'
CD14	*CD14*	gi|118129882	314-477	163	Forward 5'-GCA GAT GTG GAA TTG TAC GG-3'; Reverse 5'-GCT CCG AAT AGA ATC CGA CT-3'
F4/80	*Emr1*	gi|183583543	2109-2235	126	Forward 5'-TTG GCC AAG ATT CTC TTC CT-3'; Reverse 5'-TCA CTG CCT CCA CTA GCA TC-3'
IL-1β	*Il1b*	gi|118130747	197-388	191	Forward 5'-TGA TGA GAG CAT CCA GCT TC-3'; Reverse 5'-CAT GAG TCA CAG AGG ATG GG-3'
IL-2	*Il2*	gi|31982837	221-361	140	Forward 5'-CCT GAG CAG GAT GGA GAA TTA CA-3'; Reverse 5'-TCC AGA ACA TGC CGC AGA G-3'
IL-6	*Il6*	gi|13624310	564-718	154	Forward 5'-TGG AGT CAC AGA AGG AGT GGC TAA G-3'; Reverse 5'-TCT GAC CAC AGT GAG GAA TGT CCA C-3'
IL-8	*Il8*	gi|141802720	116-224	108	Forward 5'- ATG GCT GGG ATT CAC CTC AA-3'; Reverse 5'- AAG CCT CGC GAC CAT TCT T-3'
MCP1	*Ccl2*	gi|141803162	210-341	131	Forward 5'-TGA TCC CAA TGA GTA GGC TGG AG-3'; Reverse 5'-ATG TCT GGA CCC ATT CCT TCT TG-3'
TNFα	*Tnf*	gi|133892368	440-646	206	Forward 5'-TCG TAG CAA ACC ACC AAG TG-3'; Reverse 5'-AGA TAG CAA ATC GGC TGA CG-3'
Resistin	*Retn*	gi|21687157	121-316	196	Forward 5'-AAC TCC CTG TTT CCA AAT GC-3'; Reverse 5'-GGG CTG CTG TCC AGT CTA TC-3'
Adiponectin	*ADIPOQ*	gi|87252710	378-544	166	Forward 5'-CAC TGT TCC CAA TGT ACC CA-3'; Reverse 5'-CCT TCT TGA AGA GGC TCA CC-3'
Cyclooxygenase-1	*Ptgs1*	gi|144227245	255-431	176	Forward 5'-TAC TAT CCG TGC CAG AAC CA-3'; Reverse 5'-TCC ATG TGT CAG CAG GAA AT-3'
Cyclooxygenase-2	*Ptgs2*	gi|118130137	2912-3061	149	Forward 5'-AAG ACT TGC CAG GCT GAA CT-3'; Reverse 5'-CTT CTG CAG TCC AGG TTC AA-3'
Arachidonate 5-lipoxygenase	*Alox5*	gi|116686109	1129-1294	165	Forward 5'-CAT CCA GCT CAA CCA AAC C-3'; Reverse 5'-ACC AAA CAC CTC AGA CAC CA-3'
Arachidonate 8-lipoxygenase	*Alox8*	gi|133893229	1703-1860	157	Forward 5'-GTT CAG GCC AGT TCG ACT CT-3'; Reverse 5'-CAG CCA GAG AGC AAT GAT GT-3'
Arachidonate 12-lipoxygenase	*Alox12*	gi|145966724	1479-1631	152	Forward 5'-CAC TGC CAG GTA TGT GAA GG-3'; Reverse 5'- GAC TGG AAG GAG ACA GGG AA-3'
Arachidonate 15-lipoxygenase	*Alox15*	gi| 134948632	1433-1632	199	Forward 5'-GCA AGT CAT GAA TCG GTA CG-3'; Reverse 5'- TGA AGA TGC ACA TGG TGA TG-3'

Supplementary Table 2. Lipid profiles measured in serum and tissue samples collected from different groups of animals.

Treatment group	Normal diet (24-week old)		18 weeks of high Fat diet		Overexpressing (wild type, 12-week old)		Overexpressing (Lcn2-KO, 12- week old)	
Mice group	Wild type	Lcn2-KO	Wild type	Lcn2-KO	luciferase	Lipocalin-2	luciferase	Lipocalin-2
Serum TG (mg/dL)	42.8±1.44	33.3±3.98*	73.6±4.88	70.4±6.48	39.2±9.25	37.2±8.38	36.2±6.41	39.2±9.79
Serum TC (mg/dL)	45.1±6.19	12.5±3.20*	65.4±10.31	46.5±12.33*	26.0±2.89	32.4±7.50	13.9±3.38	13.4±6.37
Serum FFA (mmol/L)	1.1±0.08	0.9±0.27	0.9±0.01	1.0±0.11	1.1±0.34	0.92±0.32	1.0±0.22	1.0±0.11
TG in liver ($x10^{-3}$mg/mg)	12.3±3.67	8.1±2.40	20.0±0.85	6.8±1.21*	11.2±5.1	12.6±1.8	10.0±5.80	10.1±2.70
TG in epididymal fat ($x10^{-3}$mg/mg)	21.1±1.63	33.1±1.66*	36.7±0.89	64.3±2.95*	15.8±0.81	9.8±1.45*	25.1±1.56	13.3±1.70*
TG in muscle ($x10^{-3}$mg/mg)	15.1±8.17	6.1±3.22*	22.0±9.78	24.6±16.3	16.3±9.21	23.3±1.4*	11.2±0.72	25.0±1.92*
FFA in liver ($x10^{-6}$mmol/mg)	1.5±0.34	1.5±0.62	4.5±0.92	3.8±0.17*	1.1±0.31	0.83±0.13	1.0±0.28	0.9±0.14
FFA in epididymal fat ($x10^{-6}$mmol/mg)	22.7±1.61	30.8±7.45*	13.0±1.67	21.0±8.71*	20.3±1.57	15.4±3.62*	27.6±3.37	17.7±3.81*
FFA in muscle ($x10^{-6}$mmol/mg)	2.6±1.12	1.4±0.59*	2.95±0.48	2.67±0.15	5.3±0.15	7.6±0.15*	3.3±0.25	4.6±0.11*
TC in liver ($x10^{-3}$mg/mg)	0.5±0.16	0.6±0.14	0.7±0.09	0.6±0.11	1.4±0.14	1.0±0.47	0.8±0.28	1.3±0.18
TC in epididymal fat ($x10^{-3}$mg/mg)	1.8±0.09	2.3±0.47*	0.5±0.19	0.8±0.09*	0.4±0.23	0.1±0.07*	0.6±0.43	0.1±0.21*
TC in muscle ($x10^{-3}$mg/mg)	2.8±0.34	1.0±0.34*	2.5±0.12	2.3±0.13	3.1±0.38	4.4±1.08*	2.1±0.28	3.8±1.02*

* $P<0.05$ versus the corresponding control mice group (n = 5-9).

Supplementary Table 3. Quantitative PCR comparison of inflammatory genes expressed in epididymal fat pads collected from high fat fed animals.

	IL6	IL8	IL2	CD14	MCP1	F4/80	TNFα	IL1β
Wild type mice	2.33± 1.25	2.57± 0.71	0.46± 0.14	1.01± 0.72	2.75± 1.41	2.28± 0.60	3.62± 2.20	0.27± 0.12
Lcn2-KO mice	2.86± 0.90	3.26± 1.12	0.62± 0.51	0.31± 0.09*	1.40± 0.60*	0.55± 0.16*	0.83± 0.48*	0.21± 0.09

Data were presented as the fold changes over the mice fed with normal chow.

* $P<0.05$ versus the wild type mice group (n = 5-9).

Supplementary Figure legends:

Supplementary Figure 1: Lipocalin-2 treatment attenuates insulin sensitivity in mice. *A,* Western Blotting was used to measure protein levels of lipocalin-2 in liver lysates. Samples were taken from wild type (left panel) and Lcn2-KO (right panel) mice two-weeks after recombinant adenovirus injection and subjected to SDS-PAGE and Western Blotting analysis using the in-house polyclonal anti-murine lipocalin-2 antibody. *B*, Serum samples taken at five days after recombinant adenovirus injection were analysed with the in-house mouse lipocalin-2 ELISA as described in Methods. * $P < 0.05$ vs day 0, n=5. At the end of 2 wks' treatment, fasting serum insulin levels, fasting glucose levels and HOMA-IR indexes were measured for wild type (C) and Lcn2-KO mice (D) injected with recombinant adenovirus encoding luciferase (rAd-luciferase) or lipocalin-2 (rAd-Lcn2) as described in Methods. * $P < 0.05$ vs rAd-luciferase group, n=5.

Supplementary Figure 2. Increased lipogenesis and decreased lipolysis in the fat tissues of mice without lipocalin-2. The epididymal fat pads were collected from mice that were fed with high fat diets for six weeks, and subjected to analyses on lipolysis (by measuring the glycerol released into the culture medium), uptake of ^{3}H-palmitate, and lipogenesis (D-[3-3H] glucose labeling and lipid extraction). * $P < 0.05$ versus vehicle-treated samples derived from wide type mice; # $P < 0.05$ versus vehicle treated Lcn2-KO mice, n=6.

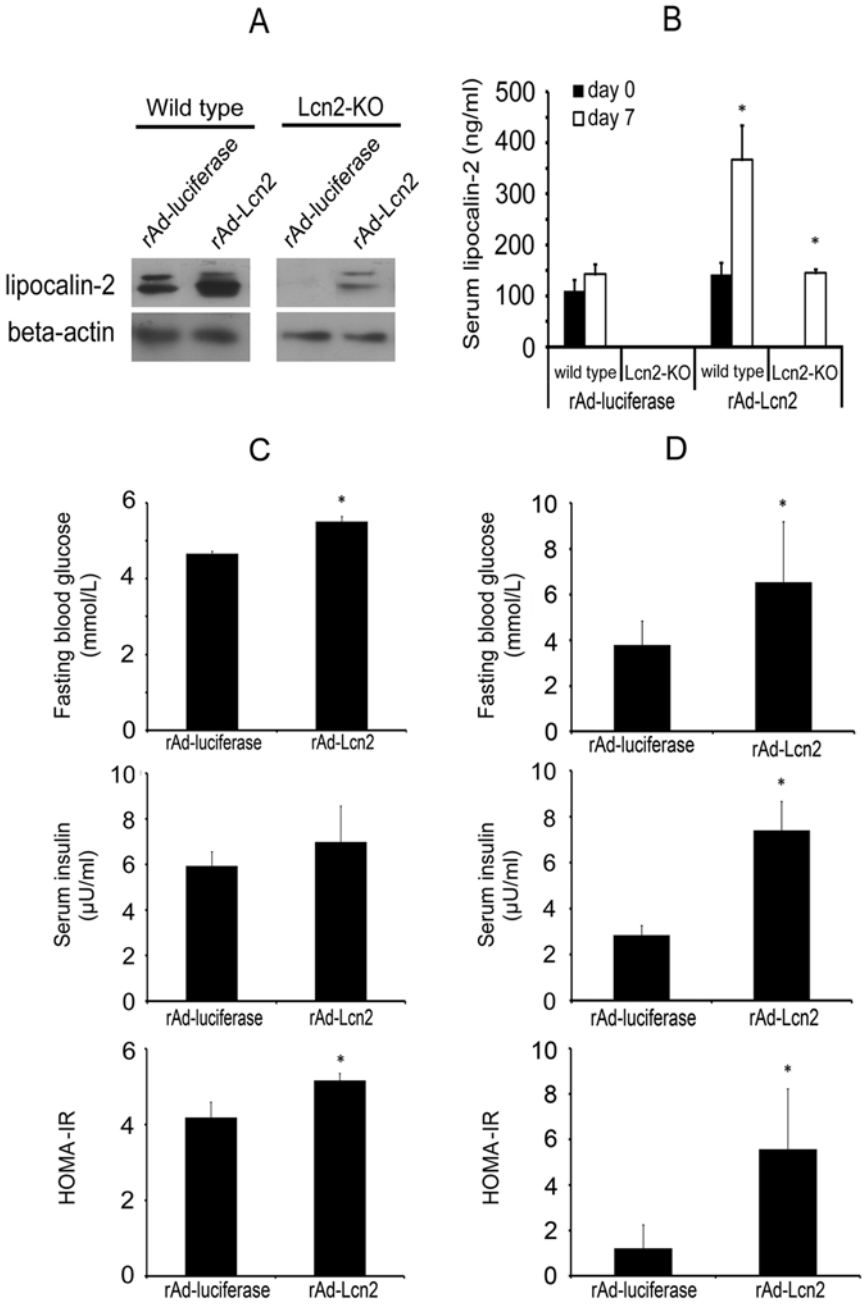
Supplementary Figure 3: Analysis of the tissue insulin sensitivity in liver and skeletal muscle of wild type and Lcn2-KO mice. *A,* Tissues were collected from normal chow, or high fat diet fed wild type and Lcn2-KO mice that were acutely injected with insulin as described in Methods. Both basal and insulin-stimulated phosphorylations of IRbeta and Akt were evaluated by Western Blotting analysis. *B,* Insulin-stimulated glucose uptake was measured in

isolated soleus muscles from wild type and Lcn2-KO mice under either normal chow or high fat diet feeding. *C,* Quantitative PCR analysis of the gene expression levels of glucose 6-phosphatase (G6P) and phosphoenolpyruvate carboxykinase (PEPCK) in the liver tissues of wild type and Lcn2-KO mice under normal chow or high fat diet feeding. Fold changes were calculated against those of normal chow-fed wild type mice. * $P < 0.05$ vs wild type mice of the same group, n=3-6.

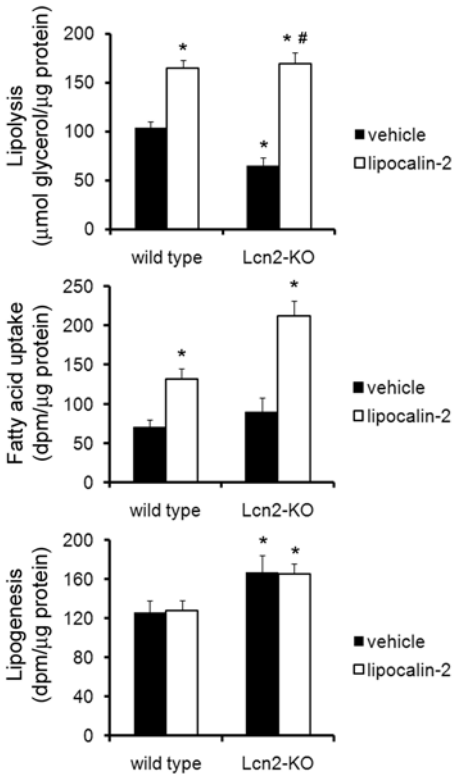
Supplementary Figure 4. GC-MS analysis of fatty acid compositions in lipid extracts from epididymal adipose, liver and soleus skeletal muscle. 25 mg epididymal adipose tissue, liver and muscle were homogenized in a mixture of 0.5 ml 4% methanolic HCl, 0.5 ml methanol and 125 µl hexane. After the addition of internal standard I (5 µl, 20 mg/ml nonadecanoic acid C19:0), the mixture was boiled at 100°C for 60 minutes and mixed with 1ml hexane and 1ml of deionized H_2O. The upper layer was collected and the internal standard II (5 ul, 10 mg/ml tridecanoic acid methyl ester methyl C13:0) was added. The volume of the samples was reduced by evaporation under nitrogen flow. Methyl esters were analyzed by gas chromatography in a 6890N Network GC system (Agilent) attached with 5973 Network Mass Selective Detector (Agilent) on a 30 m bonded 5% phenyl, 95% dimethylpolysiloxane (30 m x 0.2 mm inner diameter x 0.25 µm film thickness) HP-5ms column (Agilent). Carrier gas is helium and the flow was maintained at 0.9 ml/min and the temperature was programmed from 130°C (2 min isothermal), 140°C at 4°C/min to 200°C, 200°C at 3°C/min to 240°C, 240°C at 4°C/min to 260°C (10 min isothermal). The concentration of each fatty acid species was quantified by relating its peak area to the area of the fatty acid standard (Supelco® 37 Component FAME mix, 10mg/ml).

Supplementary Figure 5. Measurement of iron contents in adipose tissues by inductively coupled plasma-mass spectrometry analysis. The sum of stable ^{54}Fe, ^{56}Fe, ^{57}Fe, ^{58}Fe isotopes were calculated by referring to the $FeCl_3$ standard curve. Adipose tissues were collected from 24-week old mice that were fed with either standard chow or high fat diets. *, $P < 0.05$ vs wide type mice fed with standard chow, n=6.

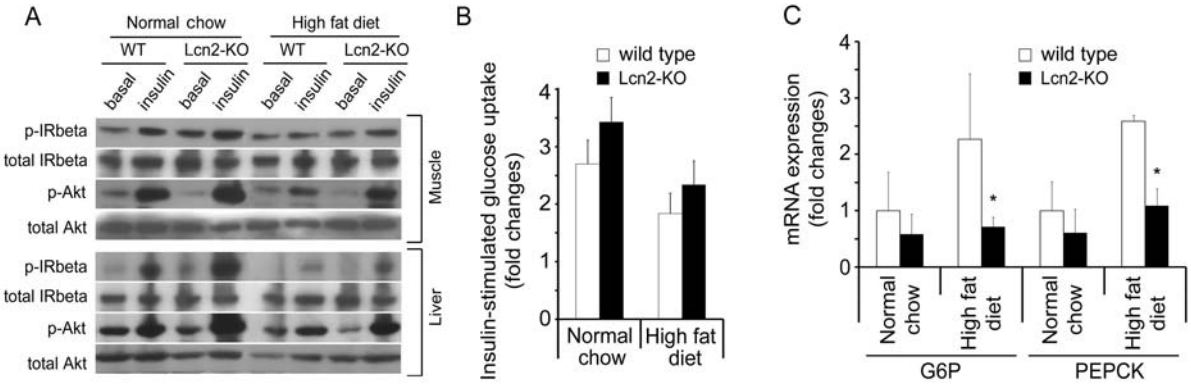
Supplementary Figure 1



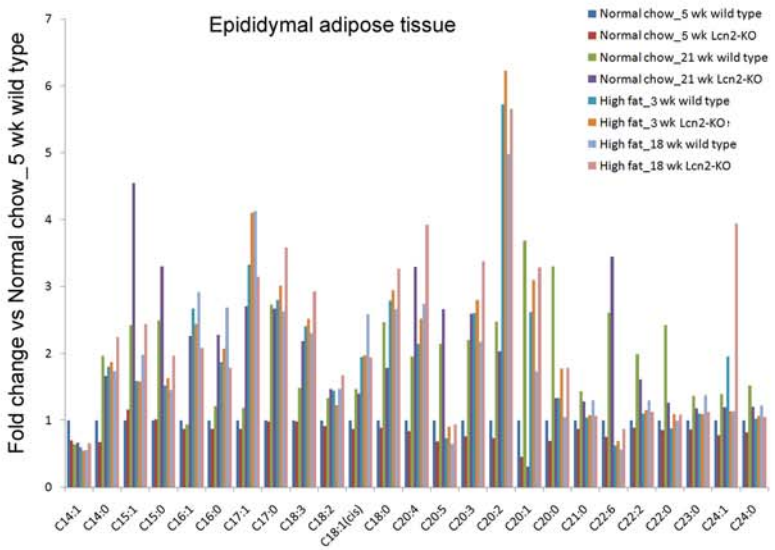
Supplementary Figure 2

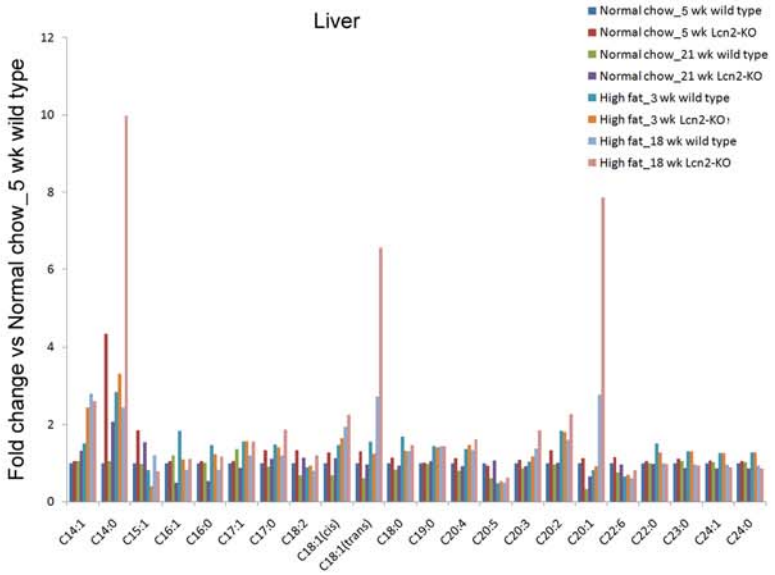


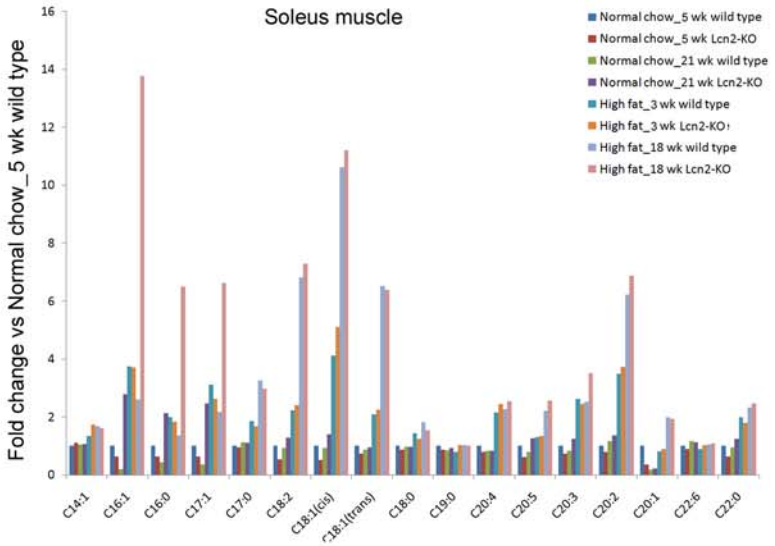
Supplementary Figure 3



Supplementary Figure 4







Supplementary Figure 5