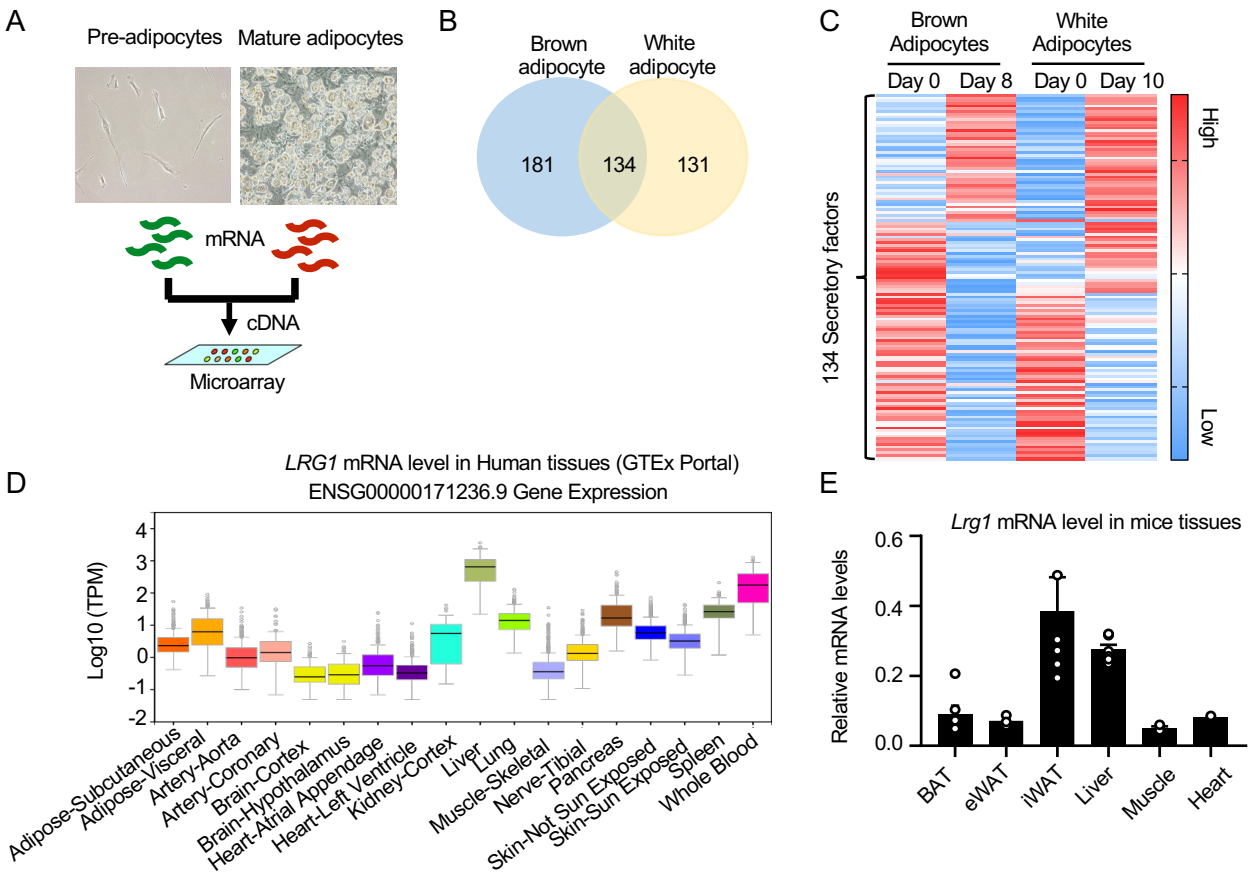
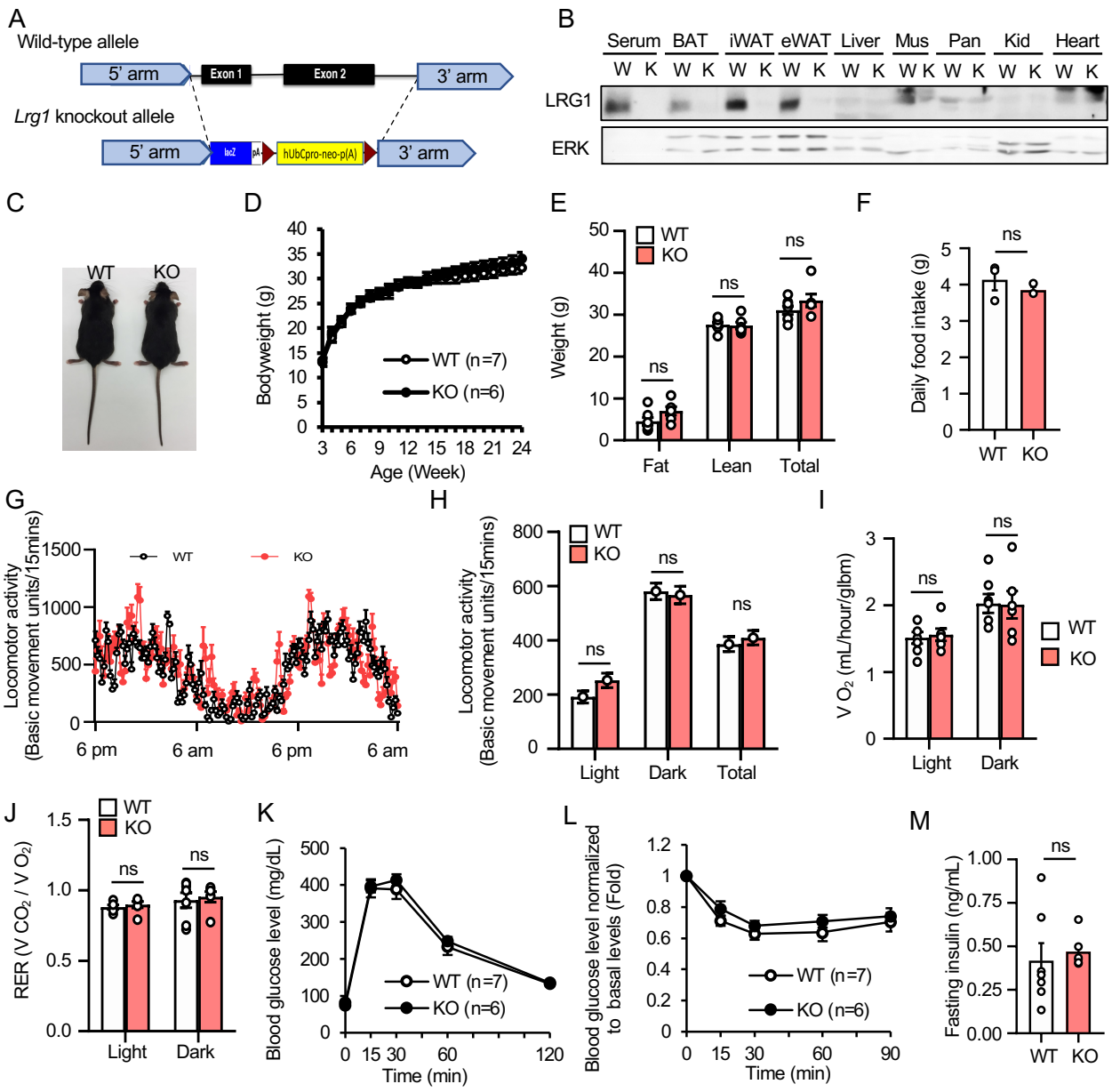
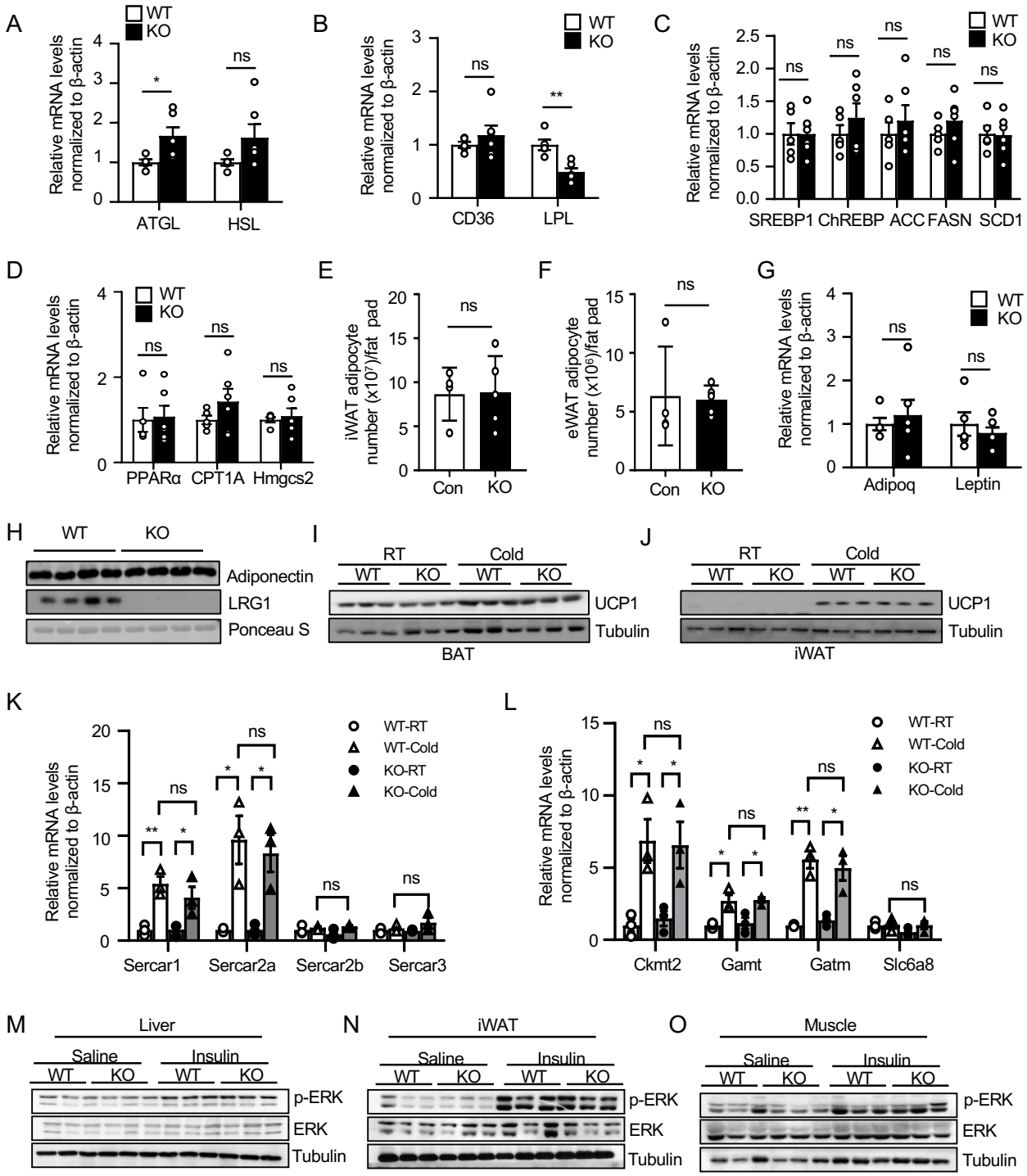


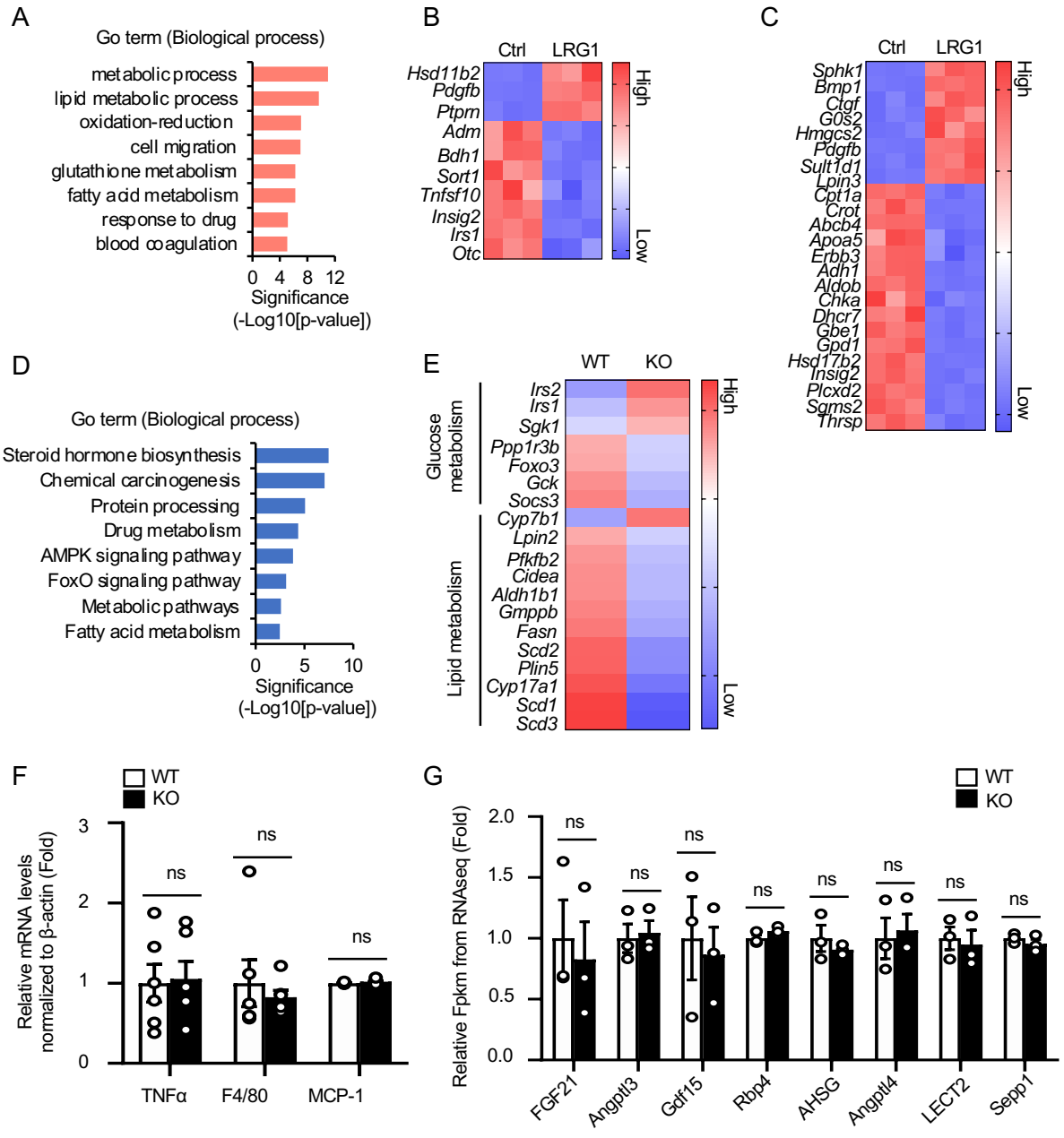
Supplemental Figure 1. DNA microarray screening for adipokines from adipocyte cell lines. (**A**) Schematic diagram for adipokine screening. Brown or 3T3-L1 pre-adipocytes (Day 0) were differentiated and harvested at Day 8 or Day 10 respectively. Gene profiling was performed by microarray expression analysis using GeneChip 3'IVT Express Kit (n=3/treatment group). (**B**) Venn diagram showing the numbers of secretory proteins with significant expression changes during brown and 3T3-L1 cell differentiation. Genes significantly altered during adipocyte differentiation ($p \leq 0.05$, fold change ≥2) were subjected to subcellular location analysis using MetazSecKB database, secretory proteins were sorted out and compared between cell lines. (**C**) Expression levels of the identified 134 secretory factors during both adipocyte cell lines differentiation. High and low levels represent the value of Z-score. (**D**) *LRG1* mRNA expression in major organs of human tissues. Data was adapted from GTEx Portal (https://gtexportal.org/home/). (**E**) *Lrg1* mRNA expression in major organs of 4-month-old C57BL/6J male mouse (n=3~6 per organ). Data represent mean ± SEM.



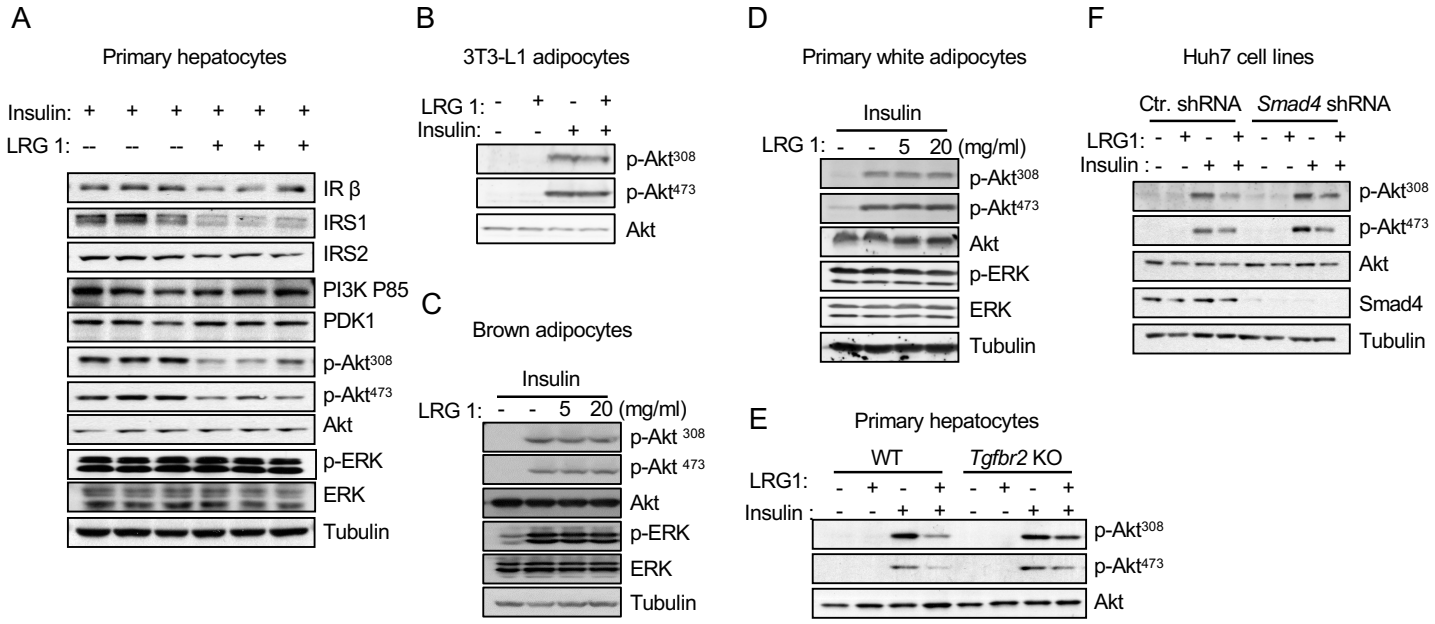
Supplemental Figure 2. *Lrg1* knockout mice and wild-type littermates exhibited similar metabolic phenotypes under normal chow feeding condition. (**A**) Schematic diagram showing the strategy for generating *Lrg1* whole-body knockout mice (*Lrg1*^{KO}). (**B**) LRG1 protein levels in the serum and tissues of wild type and homozygous *Lrg1*^{KO} mice (4-month-old male littermates). The blot is a representative of 3 independent experiments with similar results. (**C**) Appearance of 2-month-old male wild type and homozygous *Lrg1*^{KO}. (**D**) Bodyweight of male *Lrg1*^{KO} mice and control littermates under chow diet (WT: n=7, KO: n=6). (**E**) Lean and fat mass of *Lrg1*^{KO} mice and control littermates under chow diet (male, 6-month-old, WT: n=7, KO: n=6). (**F**) Daily food intake of *Lrg1*^{KO} mice and control littermates under chow diet (male, n=3 per group). (**G**) Locomotor activity of *Lrg1*^{KO} mice and control littermates under chow diet (male, 6-month-old, WT: n=7, KO: n=6). (**H**) Quantification of locomotor activity of mice in light/dark cycle. (**I**) Oxygen consumption, and (**J**) Respiratory exchange ratio (RER) of *Lrg1*^{KO} mice and control littermates under chow diet (male, 6-month-old, WT: n=7, KO: n=6). (**K**) Glucose tolerance test, (**L**) Insulin tolerance test, and (**M**) Overnight fasting serum insulin levels of *Lrg1*^{KO} mice and control littermates under chow diet conditions (male, 6-month-old, WT: n=7, KO: n=6). Data represent mean ± SEM. Unpaired two-tailed t-test, "ns (not significant)" indicate p>0.05.



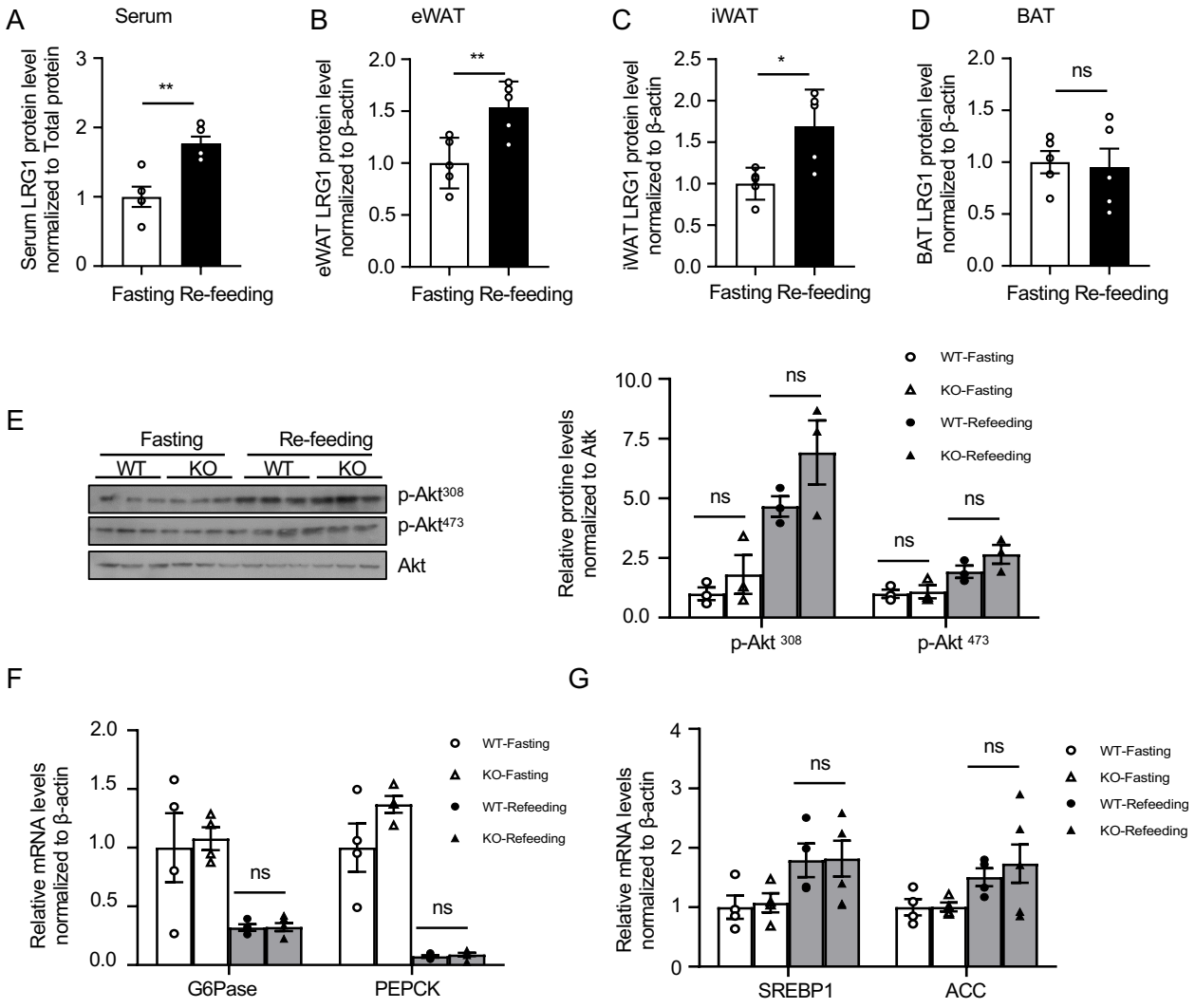
Supplemental Figure 3. Effects of *Lrg1* knockout on adipose tissue metabolism. (**A**) Lipolysis, (**B**) Lipid uptake, (**C**) Lipogenesis, and (**D**) Fatty acid oxidation gene expression in the adipose tissues of 16-week HFD-fed *Lrg1*^{KO} (n=6) and control mice (n=5) measured by qPCR. (**E**) iWAT and (**F**) eWAT adipocyte numbers from 16-week HFD-fed mice. Data are representative of 3 independent experiments. (**G**) mRNA levels of adipokines in iWAT from mice fed with 16-week HFD (n=5/genotype). (**H**) Serum adiponectin levels in 16-week HFD-fed *Lrg1*^{KO} and control mice (n=4/genotype). (**I**) BAT and (**J**) iWAT were isolated from cold stressed mice and the expression of UCP1 protein was determined by western blot (n=3/treatment group). (**K**) Ca^{2+}-dependent ATP hydrolysis thermogenic gene expression in iWAT of cold stressed mice (n=3/treatment group). (**L**) Creatine-substrate cycling thermogenic gene expression in iWAT of cold stressed mice (n=3/treatment group). Insulin-stimulated ERK signaling in mouse liver (**M**), iWAT (**N**), and skeletal muscle (**O**) from HFD-fed (16-weeks) *Lrg1*^{KO} mice and control littermates injected with insulin (1.5 U/kg bodyweight) afer fasting overnight. Mice were sacrificed 5 mins after injection and tissues were harvested for immunoblots (n=3/treatment group). Data represent mean ± SEM. Unpaired two-tailed t-test for (A) - (G), one-way ANOVA followed by Tukey's test for (K) and (L), *p≤0.05, **p≤0.01. "ns" indicate p>0.05.



Supplemental Figure 4. Impact of LRG1 on hepatic gene expression profile. (**A**) RNA-seq and biological process Go Term analysis showing most significantly altered gene expression in primary hepatocytes treated with PBS or LRG1 (20 μg/mL, 16 hours, n=3/treatment group). Total of 921 genes were significantly affected by LRG1 treatment (381 up-regulated, 540 down-regulated) using the cut off value of 2-fold and $p \leq 0.05$. Heatmaps of the insulin response genes (**B**) and lipid metabolism related genes (**C**) that were significantly altered from the RNA-seq data shown in (A). (**D**) RNA-seq and Go Term analysis showing most significantly altered biological processes in liver tissue of WT or *Lrg1*^{KO} mice after 16-week HFD feeding (n=1/treatment group). Total of 802 genes were affected by LRG1 knockout (381 up-regulated, 421 down-regulated) using the cut off value of 1.5-fold. (**E**) Heatmap of glucose and lipid metabolism related genes from the RNA-seq data in (D). (**F**) qPCR measurement of inflammatory gene expression (n=6-10/treatment group) and (**G**) Hepatokine gene expression levels (n=3/treatment group) in the liver of WT or *Lrg1*^{KO} mice after 16-week HFD feeding . Data represent mean ± SEM. Unpaired two-tailed t-test, "ns" indicate $p>0.05$.



Supplemental Figure 5. LRG1 selectively regulates insulin signaling in hepatocytes through a TGF β signaling-independent mechanism. (**A**) The phosphorylation and/or protein expression of insulin signaling components in primary hepatocytes treated with or without LRG1 (20 μg/mL, 16 h), followed by insulin (10 nM, 5 min). The effects of LRG1 on Insulin signaling in differentiated 3T3-L1 adipocytes (**B**), mouse brown adipocytes (**C**), and differentiated mouse primary white adipocytes (**D**). Cells were treated with or without LRG1 at indicated dosage for 16 hours, followed by insulin treatment (10 nM, 5 min). (**E**) Insulin-stimulated Akt phosphorylation in primary hepatocytes isolated from wild type or liver-specific TGFβ receptor II knockout mice treated with or without LRG1 (20 μg/mL) for 16 hours, followed by 10 nM insulin treatment for 5 min. (**F**) Insulin signaling in the normal and Smad4 knockdown Huh7 liver cancer cells. Cells were treated with LRG1 (20 μg/mL) for 16 hours, followed by 10 nM insulin for 5 min. Insulin-stimulated Akt phosphorylation and protein expression were determined by western blot using specific antibodies as indicated. All cell experiments were independently repeated for 3 times.



Supplemental Figure 6. Induction of LRG1 by refeeding does not affect insulin signaling and action in the liver. Male C57BL/6J mice (4-month-old, n=5/group) were fasted for 16 hrs followed by refeeding with or without NC for 2 hrs. Relative LRG1 protein levels in the serum (**A**), eWAT (**B**), iWAT (**C**), and BAT (**D**) of the mice were determined by western blots. Insulin signaling (n=3/treatment group) (**E**), gluconeogenic gene expression (n=4-6/treatment group) (**F**), and lipogenic gene expression (n=4-6/treatment group) (**G**) in the livers of these mice were determined by western blot or qPCR. Data represent mean ± SEM. Unpaired two-tailed t-test for (A) - (D). One-way ANOVA followed by Tukey's test for (E) - (G). *p≤0.05, **p≤0.01, "ns" indicate p>0.05.

SUPPLEMENTARY TABLES

Supplemental Table 1: List of primers used.

Name	5'-3' Sequence	Name	5'-3' Sequence
Human Lrg1_F	AGAGCTTTCAGGCCGTGTAG	mouse Hmgcs2_F	ATACCACCAACGCCTGTTATGG
Human Lrg1_R	CCCTGGACACCCTGGTATTG	mouse Hmgcs2_R	CAATGTCACCACAGACCAC
Human β-Actin_F	GTCATTCCAAATATGAGATGCGT	mouse HSL_F	TGTGTCAGTGCCTATTCAG
Human β-Actin_R	GGTCAGACAGCTTGCGGAT	mouse HSL_R	GAACAGCGAAGTGTCTCT
mouse ACC_F	CCACAATGATCCTCCGAATCC	mouse IL-6_F	TTCCAATGCTCTCCTAACA
mouse ACC_R	ATACTCAGGACTCTCAAACTAAGC	mouse IL-6_R	GTCCACAAACTGATATGCTTA
mouse Adipoq_F	CCACAATGATCCTCCGAATCC	mouse IRS1_F	TCCTATCCCGAAGAGGGTCT
mouse Adipoq_R	ATACTCAGGACTCTCAAACTAAGC	mouse IRS1_R	TGGGCATATAGCCATCATCA
mouse ApoB_F	ACCAAGCTGGCATAAGAACCA	mouse IRS2_F	CACAATTCCAAGCGCCACAA
mouse ApoB_R	CCTCCATCCTGAGTTGGACA	mouse IRS2_R	CATCACCTCCTCCCAGGGTA
mouse ATGL_F	GCTGTGGAATGAGGACATAGGA	mouse Leptin_F	CAAGCAGTGCCTATCCAGA
mouse ATGL_R	GCATAGTGAGTGGCTGGTGAA	mouse Leptin_R	AAGCCCAGGAATGAAGTCCA
mouse CD36_F	ATTCCCTTGGCAACCAACCA	mouse LPL_F	GGCTGACACTGGACAAACAAA
mouse CD36_R	TACGTGGCCCGGTTCTACTA	mouse LPL_R	CCTGGGTTAGCCACCGTTTA
mouse ChREBP_F	CTGGGGACCTAAACAGGAGC	mouse Lrg1_F	CAGATTCCTCATTCCCTCAG
mouse ChREBP_R	GAAGCCACCCTATAGCTCCC	mouse Lrg1_R	CGTGTCAAAGCCAGATAAAC
mouse Ckmt1_F	TGAGGAGACCTATGAGGTATTTGC	mouse MCP1_F	GCATCCACGTGTTGGCTCA
mouse Ckmt1_R	TCATCAAAGTAGCCAGAACGGA	mouse MCP1_R	CTCCAGCCTACTCATTGGGATCA
mouse CPT1A_F	CCAGGCTACAGTGGGACATT	mouse MTTP_F	AGCGTTGCATTTCTACCCAC
mouse CPT1A_R	GAACTTGCCCATGTCCTTGT	mouse MTTP_R	GCCAACACGTCTAGCCAGTA
mouse Cyp7A1_F	GATTTAGGAAGGCCCGGAGG	mouse PEPCK_F	GGCCACAGCTGCTGCAG
mouse Cyp7A1_R	TGGAATAAGGAGAAGGCATTTGGA	mouse PEPCK_R	GGTCGCATGGCAAAGGG
mouse F4/80_F	CTTTGGCTATGGGCTTCCAGTC	mouse PPARα_F	TCGCTATCCAGGCAGAAG
mouse F4/80_R	GCAAGGAGGACAGAGTTTATCGTG	mouse PPARα_R	ACCACAGACCAACCAAGT
mouse FABP1_F	CCCGAGGACCTCATCCAGAA	mouse SCD1_F	CTGTACGGGATCATACTGGTTC
mouse FABP1_R	CCCCAGGGTGAACTCATTGC	mouse SCD1_R	GCCGTGCCTTGTAAGTTCTG
mouse FASN_F	GCGATGAAGAGCATGGTTTAG	mouse Sercar1_F	TGTTTGTCCTATTTCGGGGTG
mouse FASN_R	GGCTCAAGGGTTCCATGTT	mouse Sercar1_R	AATCCGCACAAGCAGGTCTTC
mouse FATP2_F	TTTCCGGTGGAAAGGAGA	mouse Sercar2a_F	GCTCATTTTCCAGATCACACCG
mouse FATP2_R	AGGTGCTCCTGATGTGTTG	mouse Sercar2a_R	GTTACTCCAGTATTGCGGGTTG
mouse G6Pase_F	AGCCTCCGGAAGTATTGTCTCA	mouse Sercar2b_F	ACCTTTGCCGCTCATTTTCCAG
mouse G6Pase_R	TCCACCCCTAGCCCTTTTAGTAG	mouse Sercar2b_R	AGGCTGCACACACTCTTTACC
mouse Gamt_F	GCAGCCACATAAGGTTGTTCC	mouse Sercar3_F	GGAGCAGTTTGAGGACCTCTT
mouse Gamt_R	CTCTTCAGACAGCGGGTACG	mouse Sercar3_R	GGCCACGAGAATTAGCATGATG
mouse Gatm_F	GACCTGGTCTTGTGCTCTCC	mouse Slc6a8_F	TGCATATCTCCAAGGTGGCAG
mouse Gatm_R	GGGATGACTGGTGTTGGAGG	mouse Slc6a8_R	CTACAAACTGGCTGTCCAGA
mouse β-Actin_F	GTTGGTTGGAGCAAACATC	mouse SREBP1_F	CCCTGTGTGTACTGGCCTTT
mouse β-Actin_R	CTTATTTCATGGATACTTGGAATG	mouse SREBP1_R	TTGCGATGTCTCCAGAAGTG
mouse TNFα_F	AGAGAAGCAACTACAGACC		
mouse TNFα_R	CAGTATGTGAGAGGAAGAGAA		

Supplemental Table 2: List of antibodies used.

Antibody	Source	Identifier
Rabbit polyclonal anti-ACC	Cell signaling technology	Cat.# 3662
Mouse monoclonal anti-AKT	Cell signaling technology	Cat.# 2966
Rabbit polyclonal anti-Phospho-Akt (Thr308)	Cell signaling technology	Cat.# 9275S
Rabbit polyclonal anti-Phospho-Akt (Ser473)	Cell signaling technology	Cat.# 9271S
Rabbit monoclonal anti-FAS	Cell signaling technology	Cat.# 3180
Rabbit polyclonal anti-IR β	Santa Cruz	Cat.# sc711
Rabbit polyclonal anti-IRS1	Cell signaling technology	Cat.# 2382S
Rabbit polyclonal anti-IRS2	Cell signaling technology	Cat.# 4502
Rabbit polyclonal anti-p44/42 MAPK (ERK1/2)	Cell signaling technology	Cat.# 9102S
Rabbit polyclonal anti-Phospho-ERK1/2	Cell signaling technology	Cat.# 4370S
Rabbit polyclonal anti-PI 3-kinase p85alpha	Santa Cruz	Cat.# sc423
Rabbit monoclonal anti-SCD1	Cell signaling technology	Cat.# 2794S
Mouse monoclonal anti-Smad4	Santa Cruz	Cat.# sc7966
Mouse monoclonal anti-SREBP1	Abcam	Cat.# ab3259
Rabbit polyclonal anti-β-Tubulin	Cell Signaling Technology	Cat.# 2146
Rabbit polyclonal anti-UCP1	Abcam	Cat.# ab10983
Rabbit polyclonal anti-Adiponectin	Liu et al., 2018, Homemade	N/A
Rabbit polyclonal anti-PDK1	Dong et al., 1999, Homemade	N/A
Rabbit polyclonal anti-LRG1	This paper, Homemade, see methods for details of production.	N/A

Supplemental Table 3: Sources of cell lines and animal lines used.

Cell line / Mouse line	Source
Brown fat cell line	Purchased from Millipore sigma (Cat.# SCC255)
3T3-L1 cell line	Purchased from ATCC (Cat.# CL-173)
Huh7 cell line	Gift from Dr. Luzhe Sun (UTHealth SA)
Huh7 sh-smad4 cell line	Gift from Dr. Luzhe Sun (UTHealth SA)
C57BL/6J mouse line	The Jackson Laboratory (Cat.# 000664)
Lrg1 wholebody-knockout mouse line	KOMP Repository, University of California (Line# ET11851)
Leptin receptor deficient mouse line (*db/db*)	The Jackson Laboratory (Cat.# 000697)
Alb-cre mouse line (for liver-specific TGFβ receptor II knockout)	The Jackson Laboratory (Cat.# 016832)
TGFβ receptor II loxp/loxp mouse line (for liver-specific TGFβ receptor II knockout)	The Jackson Laboratory (Cat.# 012603)

SUPPLEMENTARY REFERENCES

1. Liu, M.*, et al.* A disulfide-bond A oxidoreductase-like protein (DsbA-L) regulates adiponectin multimerization. 2008; Proc Nat Acad Sci USA. 105 (47), 18302-18307.
2. Dong, L. Q., *et al*. Primary Structure, Tissue Distribution, and Expression of Mouse Phosphoinositide-dependent Protein Kinase-1, a Protein Kinase That Phosphorylates and Activates Protein Kinase Cζ. 1999; J Biol Chem. 274 (12), 8117–8122.