

Supplemental Methods

RT-PCR: RNA isolation and RT-PCR was performed as per manufacturer's instructions (Stratagene, CA, USA; Invitrogen, Burlington, Canada). Primer sequences are shown in Supplemental Table S1.

Activity and Indirect Calorimetry: Wildtype and CMKLR1^{-/-} mice fed a LF or HF diet for 6 weeks were individually housed in Panlab Physiocage 00 for 24 h for activity and indirect calorimetry analysis (Panlab, Holliston, MA, USA).

Histology: Liver samples were fixed in 10% acetate buffered formalin for 48 h and subsequently embedded in paraffin. Deparaffinized sections (5 μ M) were stained with Harris Hematoxylin and Eosin Y (Sigma Aldrich). Area fraction of lipid deposits was quantified using ImageJ.

Supplemental Figure Legends

Figure S1. Screening of mice for CMKLR1 ablation. RT-PCR screening of WAT, liver, and SKM using CMKLR1 primers (Table S1) and analysis by 2.5% agarose gel electrophoresis for a 150 bp product indicative of mRNA expression of CMKLR1. NTC = no template control. Representative images are shown.

Figure S2. Hypothalamic leptin receptor, neuropeptide Y, and agouti-related peptide mRNA levels are similar in wildtype and CMKLR1^{-/-} mice. Hypothalamic leptin receptor, neuropeptide Y, and agouti-related peptide mRNA levels as measured by quantitative real-time PCR at week 24 in wildtype and CMKLR1^{-/-} mice fed a LF or HF diet. Values are expressed as mean \pm SEM. * $p < 0.05$, comparing wildtype to CMKLR1^{-/-} mice within the diet. N=6-10 mice/group.

Figure S3. CMKLR1^{-/-} mice have lower oxygen consumption, carbon dioxide production, and energy expenditure, and a higher respiratory quotient than wildtype mice. Wildtype and CMKLR1-

/- mice fed a LF or HF diet for 6 weeks were individually housed in Panlab Physiocage 00 for 24 h with a fixed 12 h light (7:00 – 19:00):12 h dark (19:00 – 7:00) cycle to measure activity levels (**A, B**), oxygen consumption (**C, D**), carbon dioxide production (**E, F**), and respiratory quotient (**G, H**). The values are expressed as mean \pm SEM.

Figure S4. CMKLR1^{-/-} mice have less hepatic steatosis than wildtype mice. Histological analysis of liver sections (H&E staining) from week 6 and 24 wildtype and CMKLR1^{-/-} mice fed a LF or HF diet. Representative images are shown. Area fraction of lipid deposits was quantified using ImageJ software. Values are expressed as means \pm SEM. * $p < 0.05$, comparing wildtype to CMKLR1^{-/-} mice within the diet. N=6-10 mice/group.

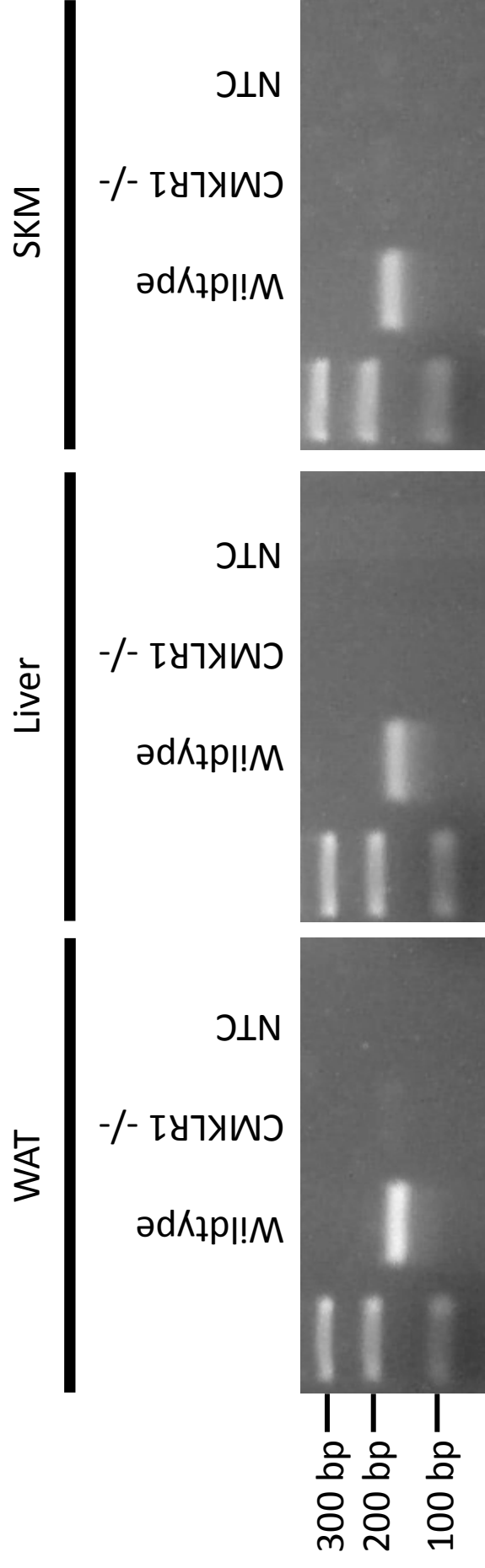
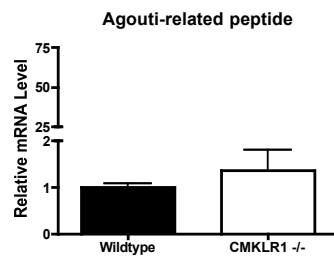
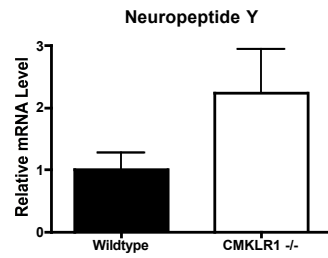
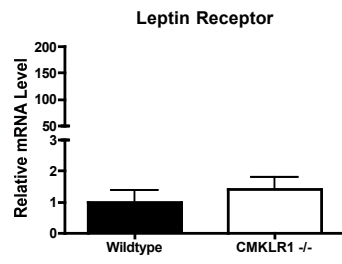
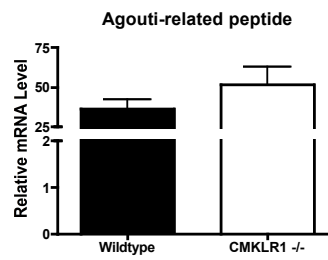
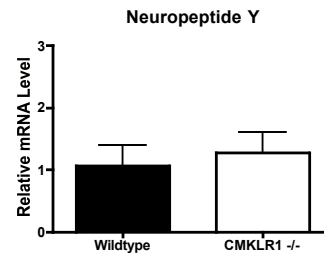
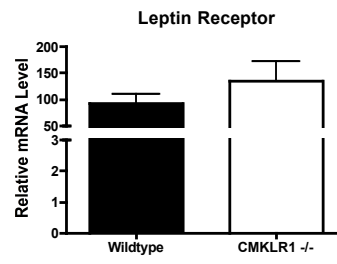


Figure S1

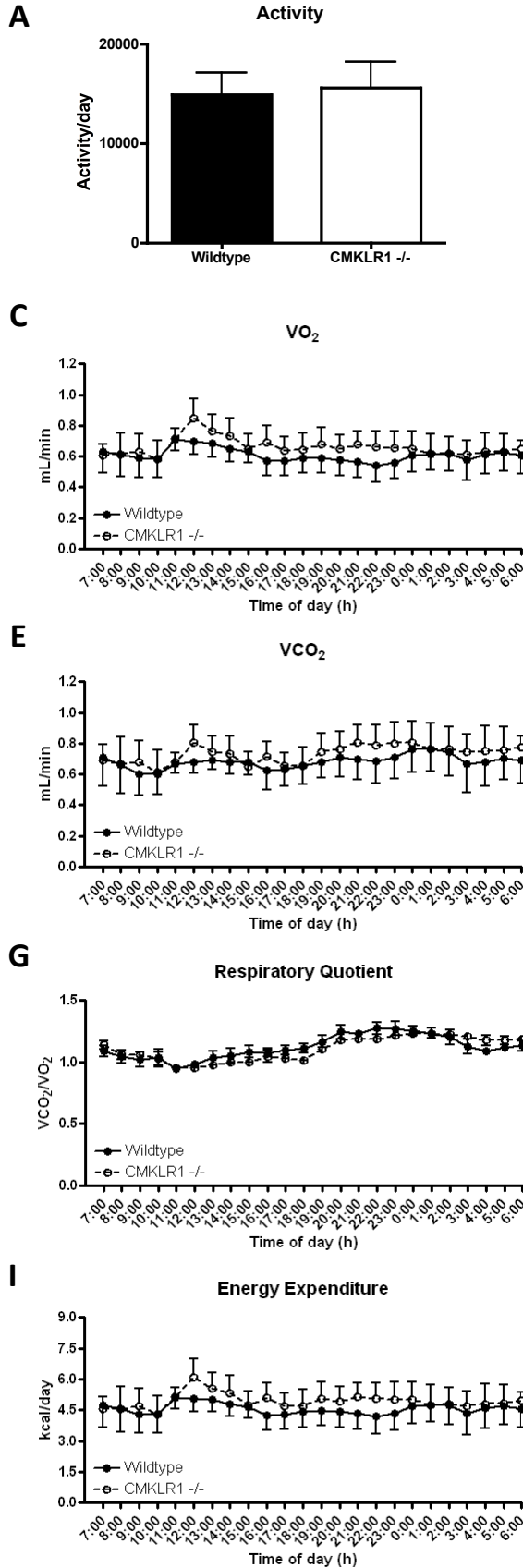
Low-fat Diet



High-fat Diet



Low-fat Diet



High-fat Diet

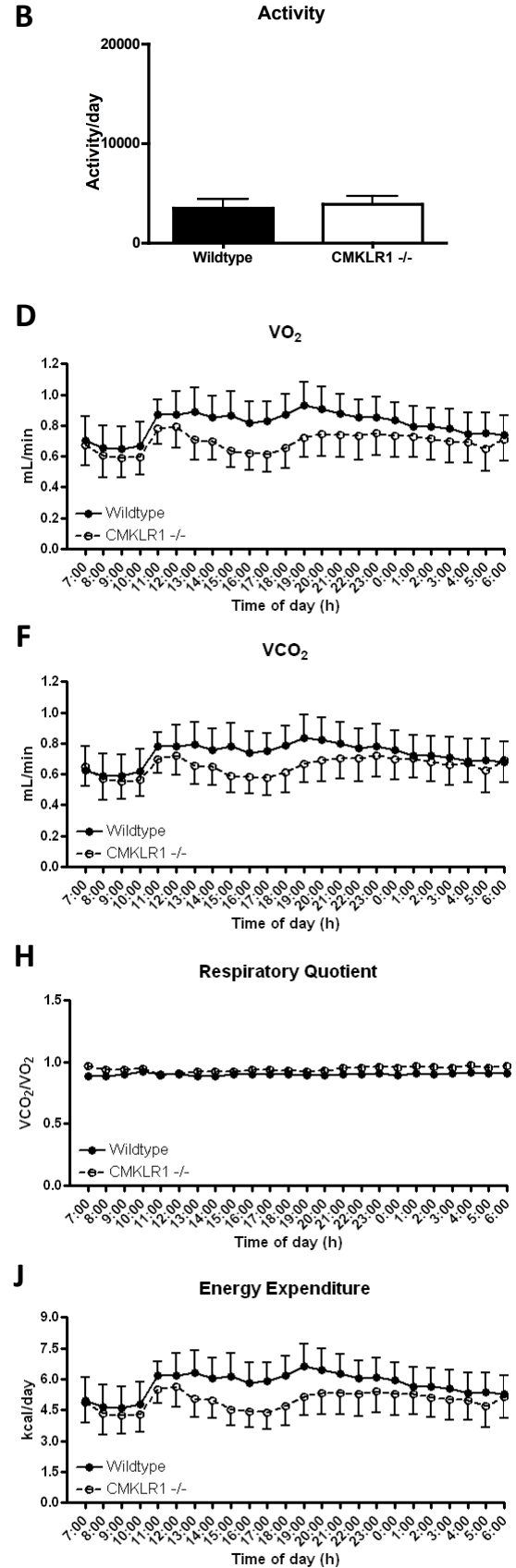
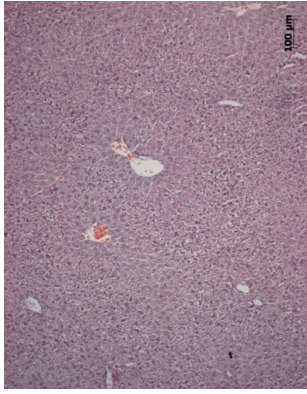


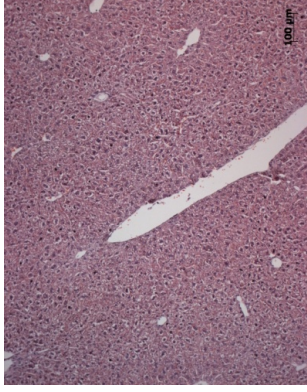
Figure S3

Low-fat Diet

Wildtype

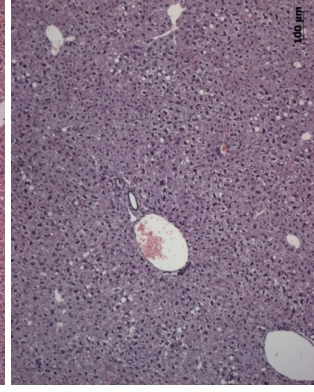
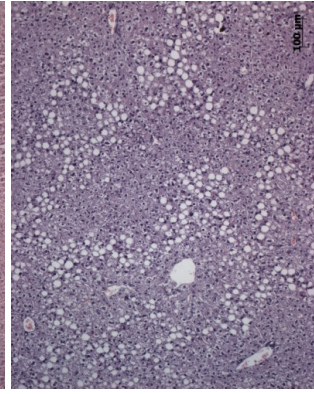


CMKLR1 -/-



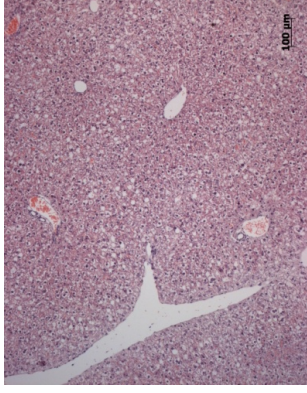
Week 6

Week 24



High-fat Diet

Wildtype



CMKLR1 -/-

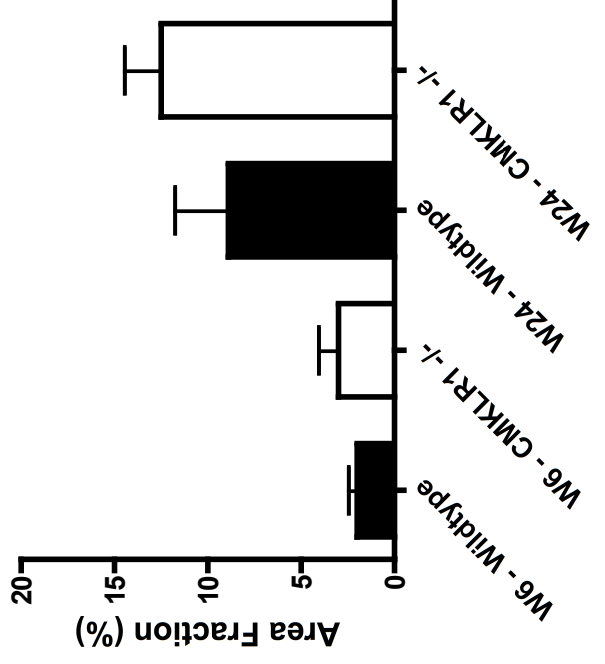
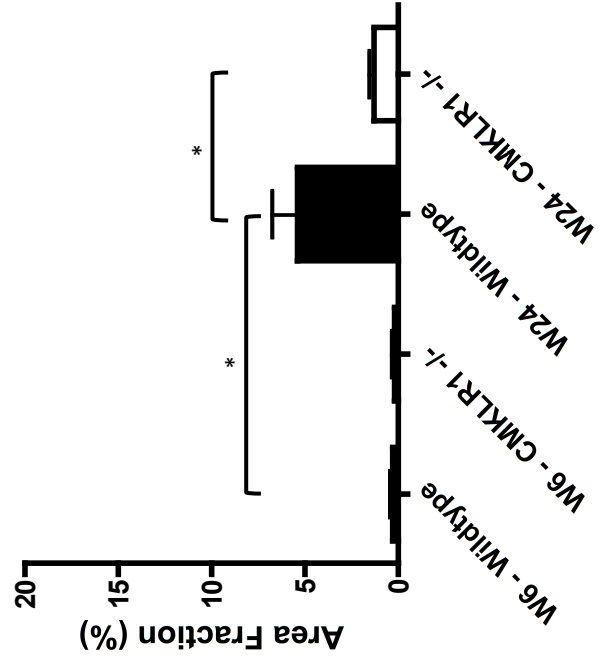
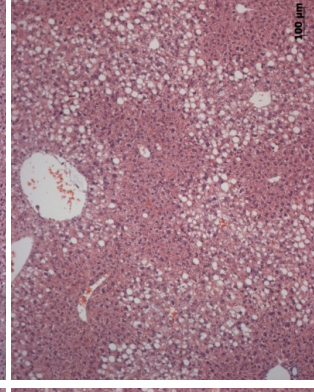
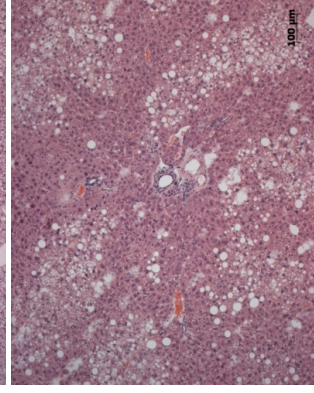
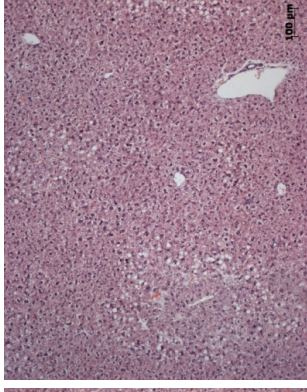


Figure S4

Table S1. Quantitative real-time PCR primer sequences

| Gene | Accession Number | Sequence (5' -> 3') |
|-----------------------|------------------|---|
| Cyclophilin | NM_008907 | Fw GAG CTG TTT GCA GAC AAA GTT C Rv CCC TGG CAC ATG AAT CCT GG |
| IL-6 | NM_031168 | Fw TAG TCC TTC CTA CCC CAA TTT CC Rv TTG GTC CTT AGC CAC TCC TTC |
| TNF α | NM_013693 | Fw CCC TCA CAC TCA GAT CAT CTT CT Rv GCT ACG ACG TGG GCT ACA G |
| CMKLR1 | NM_013693 | Fw GCT TTG GCT ACT TTG TGC ACT Rv CAG TGT TCA CGG TCT TCT TCA |
| UCP1 | NM_009463 | Fw CAC TCA GGA TTG GCC TCT ACG Rv GGG GTT TGA TCC CAT GCA GA |
| AgRP | NM_007427 | Fw GGC CTC AAG AAG ACA ACT GC Rv GCA AAA GGC ATT GAA GAA GC |
| LeptinR | NM_146146 | Fw GGT TGG ATG AGC TTT TGG AA Rv TCC TGG AGG ATC CTG ATG TC |
| NPY | NM_023456 | Fw AGA GAT CCA GCC CTG AGA CA Rv GAT GAG GGT GGA AAC TTG GA |
| Insulin Receptor | NM_010568 | Fw CCT GTA CCC TGG AGA GGT GT Rv CGG ATG ACT GTG AGA TTT GG |
| Glucose Transporter 4 | NM_012751 | Fw ACT CTT GCC ACA CAG GCT CT Rv AAT GGA GAC TGA TGC GCT CT |