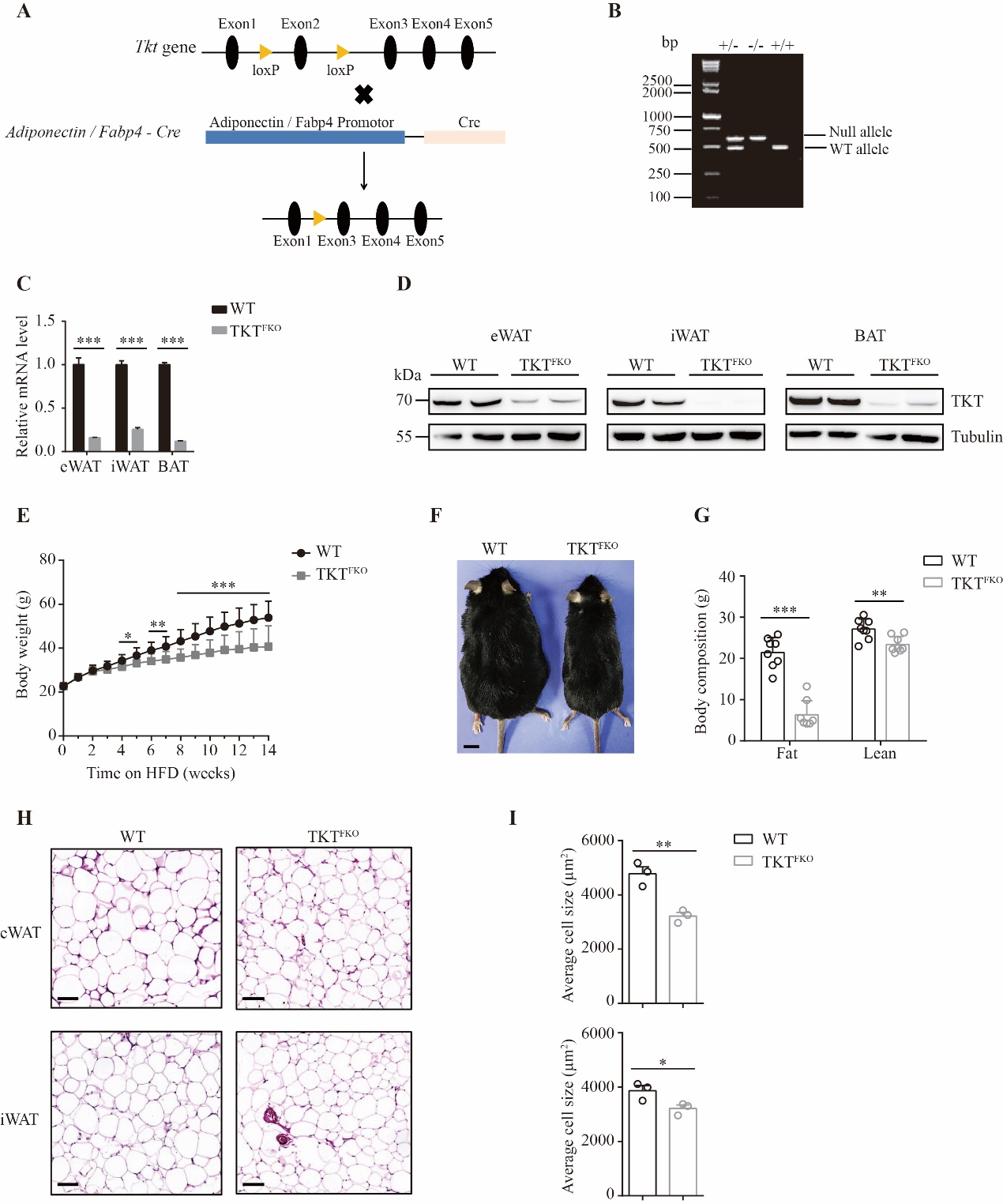
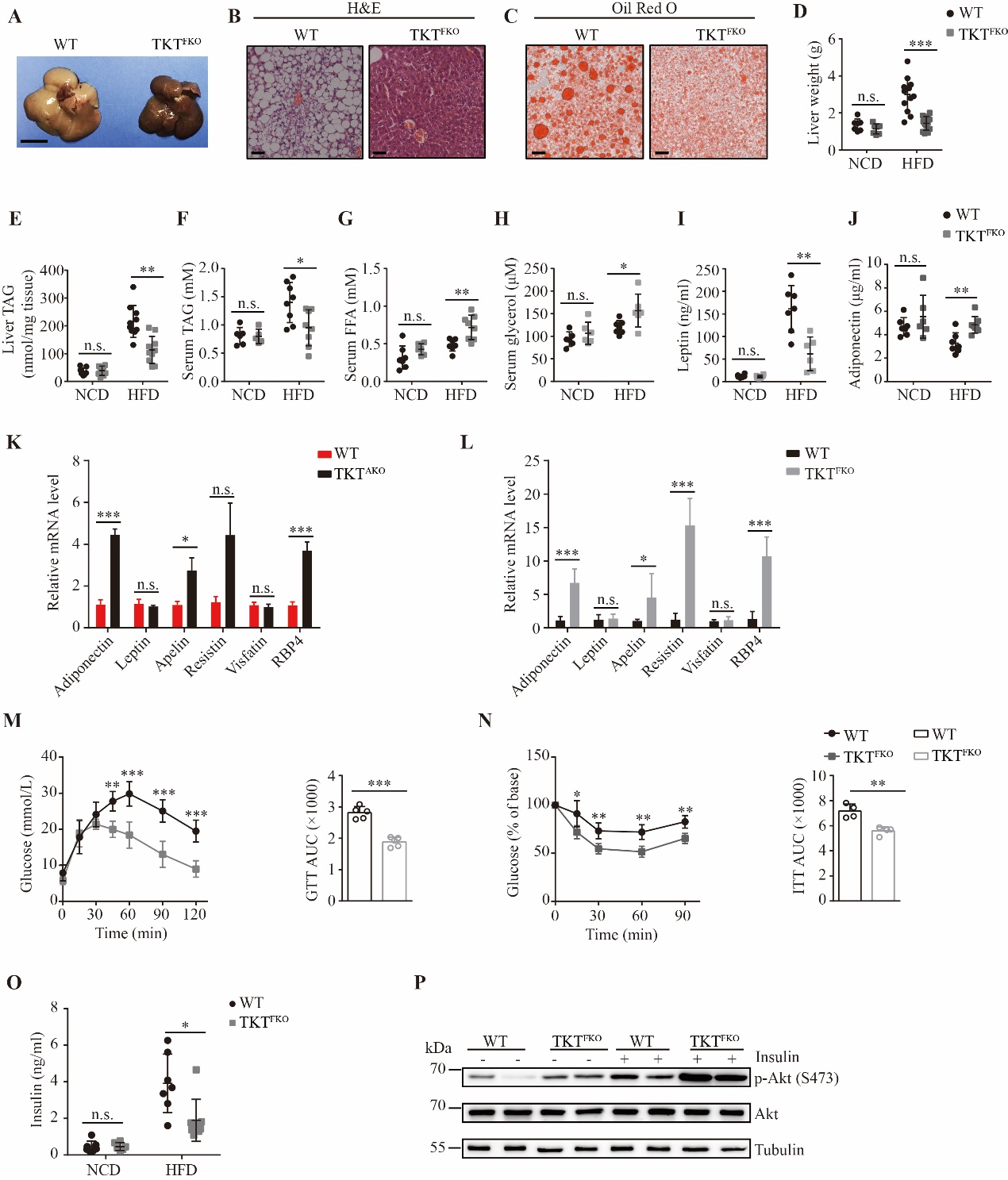
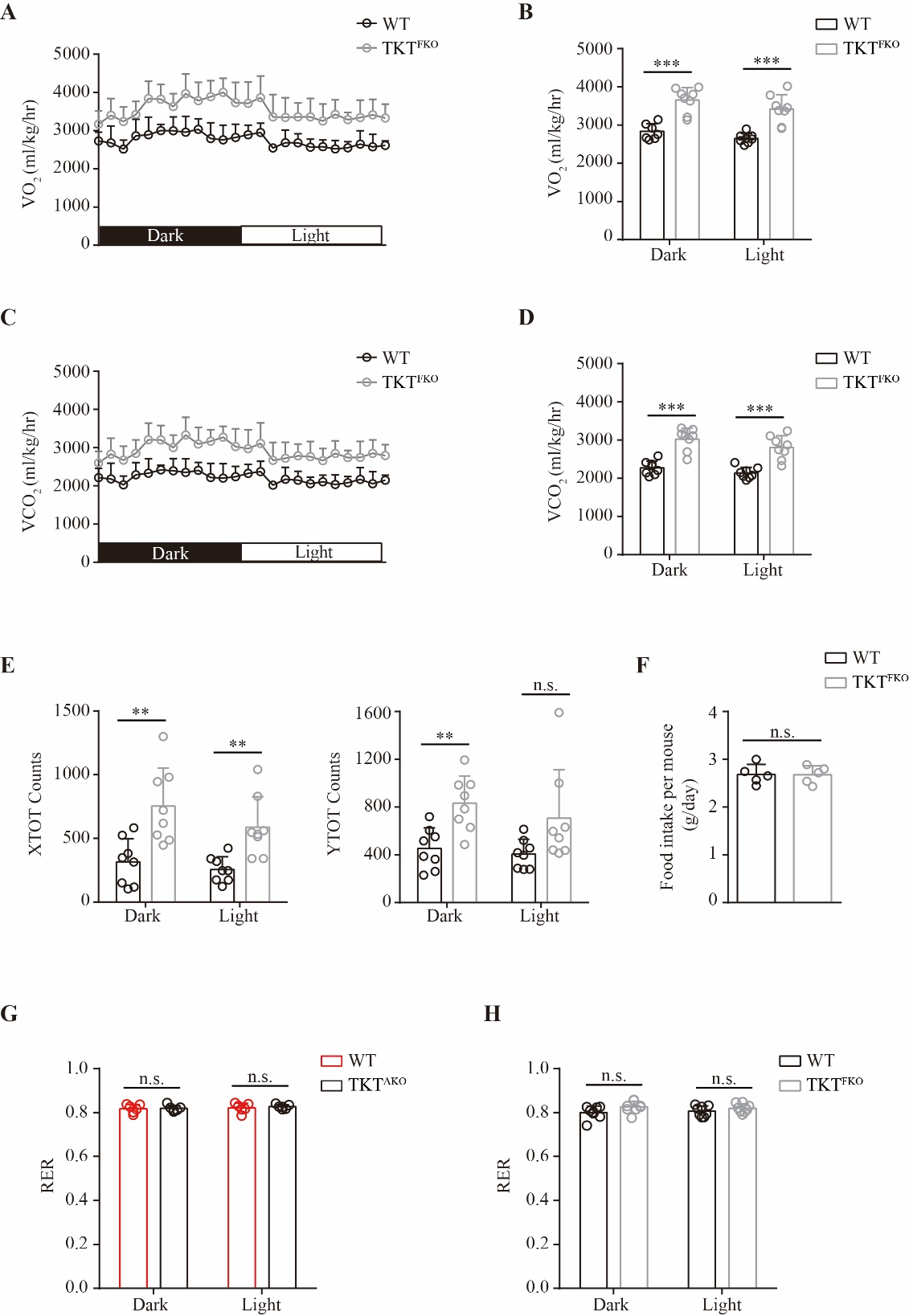
**Supplementary Figures**



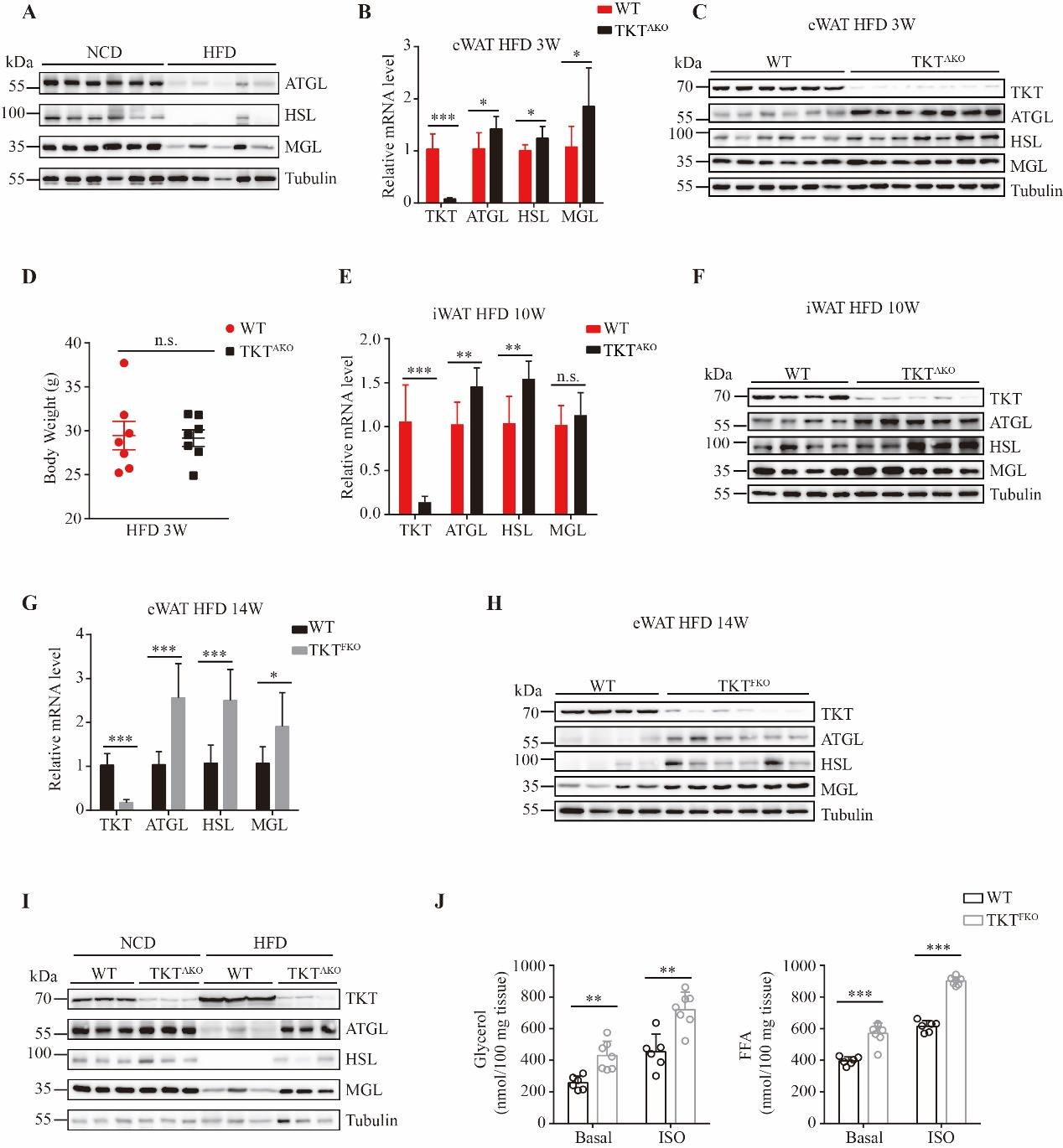
**Supplementary Fig. 1** TKT deficiency in adipocytes protects mice from HFD-induced obesity. (*A*) Schematic diagram of the strategy to generate TKTAKO and TKTFKO mice. (*B*) PCR analysis for TKT WT and null alleles. (*C*) qPCR analysis of TKT mRNA expression in adipose tissues from WT and TKTFKO mice (n=3). (*D*) Western blotting analysis of TKT protein levels in tissues from WT and TKTFKO mice. (*E*) Body weight of WT and TKTFKO mice on HFD (n=13). (*F*) Representative images of WT and TKTFKO mice on 14-week HFD from 6-week old. Scale bars, 1 cm. (*G*) Body composition of WT and TKTFKO mice on 14-week HFD from 6-week old (n=8). (*H*) Representative H&E images of eWATs and iWATs from WT and TKTFKO mice on 14-week HFD from 6-week old. Scale bars, 100 μm. (*I*) Quantification of adipocyte size (n=3). Data are represented as mean ± SEM. Significance is determined by Student’s *t* test analysis, \**P* < 0.05, \*\**P*<0.01, \*\*\**P*<0.001.



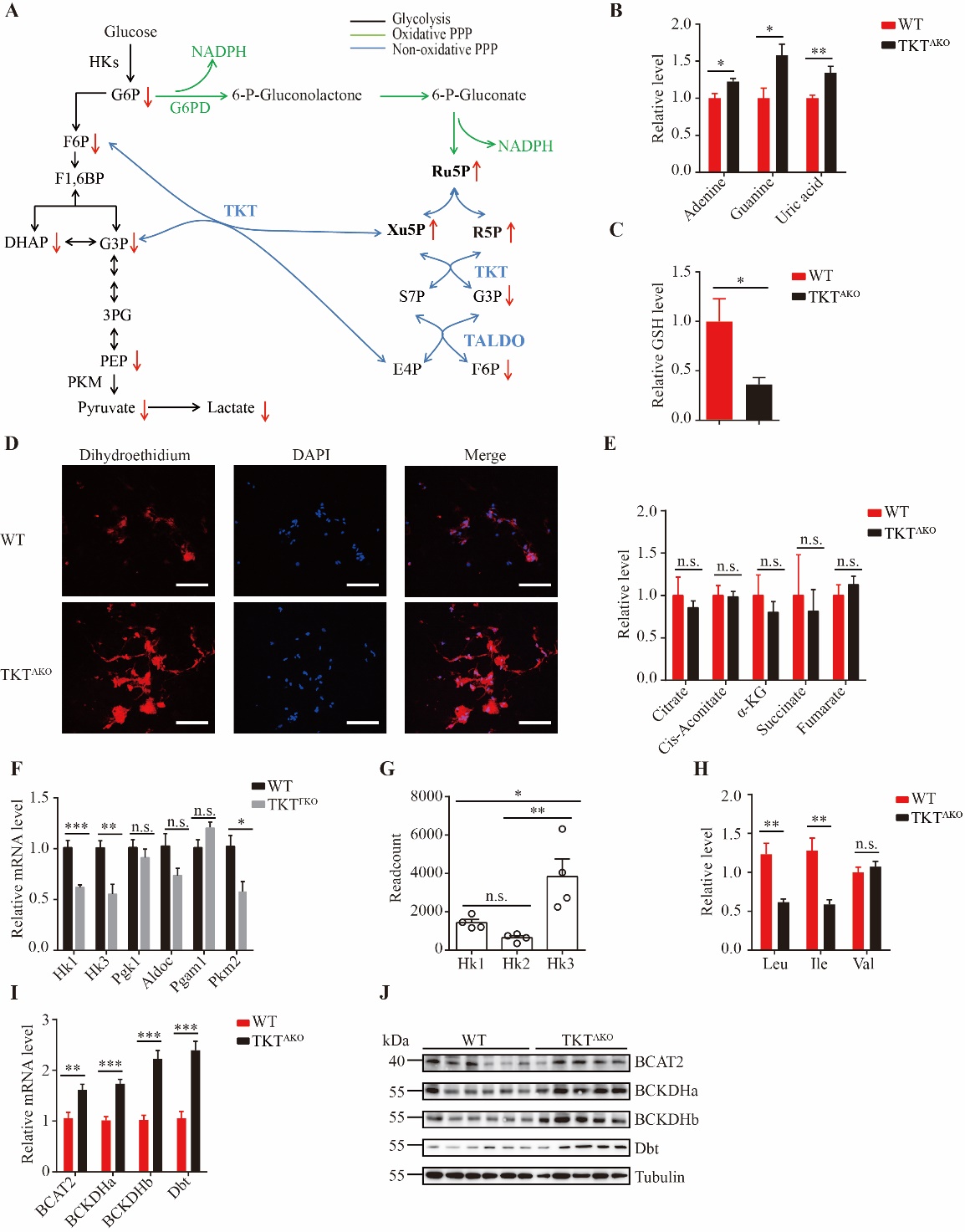
**Supplementary Fig. 2** Adipocyte-specific TKT abrogation ameliorates HFD-induced metabolic disorders. NCD represents 5-month old mice on NCD. 10- or 14-week HFD represents mice fed with HFD from 6-week old for 10 or 14 weeks, respectively. (*A*) Representative images of livers from WT and TKTFKO mice fed with 14-week HFD. Scale bars, 1 cm. (*B*) Representative images of H&E stained liver sections from WT and TKTFKO mice on 14-week HFD. Scale bars, 50 μm. (*C*) Representative images of Oil Red O stained liver sections from WT and TKTFKO mice on 14-week HFD. Scale bars, 50 μm. (*D*) Liver weight of WT and TKTFKO mice fed with NCD (n=7) and 14-week HFD (n=13). (*E*) Levels of hepatic TAG of WT and TKTFKO mice fed with NCD (n=9) and 14-week HFD (n=10). (*F*) Serum levels of TAG of WT and TKTFKO mice fed with NCD (n=6 for WT mice, n=5 for TKTFKO mice) and 14-week HFD (n=8). (*G*) Serum levels of FFA of WT and TKTFKO mice fed with NCD (n=7 for WT mice, n=6 for TKTFKO mice) and 14-week HFD (n=7 for WT mice, n=8 for TKTFKO mice). (*H*) Levels of serum glycerol of WT and TKTFKO mice fed with NCD (n=6) and 14-week HFD (n=7 for WT mice, n=6 for TKTFKO mice). (*I*) Levels of serum leptin of WT and TKTFKO mice fed with NCD (n=6) and 14-week HFD (n=7 for WT mice, n=6 for TKTFKO mice). (*J*) Serum levels of adiponectin of WT and TKTFKO mice fed with NCD (n=7 for WT mice, n=6 for TKTFKO mice) and 14-week HFD (n=7 for WT mice, n=8 for TKTFKO mice). (*K*) qPCR analysis of adipokine mRNA levels in adipose tissues from WT and TKTAKO mice fed with 10-week HFD (n=8). (*L*) qPCR analysis of adipokine mRNA levels in adipose tissues from WT and TKTFKO mice fed with 14-week HFD (n=8). (*M*) Glucose tolerance test (GTT) of WT and TKTFKO mice fed with 14-week HFD (n=5). (*N*) Insulin tolerance test (ITT) of WT and TKTFKO mice fed with 14-week HFD (n=4). (*O*) Serum levels of insulin of WT and TKTFKO mice fed with NCD (n=7 for WT mice, n=6 for TKTFKO mice) and 14-week HFD (n=7 for WT mice, n=8 for TKTFKO mice). (*P*) Western blotting analysis of Akt phosphorylation in eWATs of WT and TKTFKO mice on 14-week HFD. Data are represented as mean ± SD. Significance is determined by Student’s *t* test analysis, \**P* < 0.05, \*\**P*<0.01, \*\*\**P*<0.001.



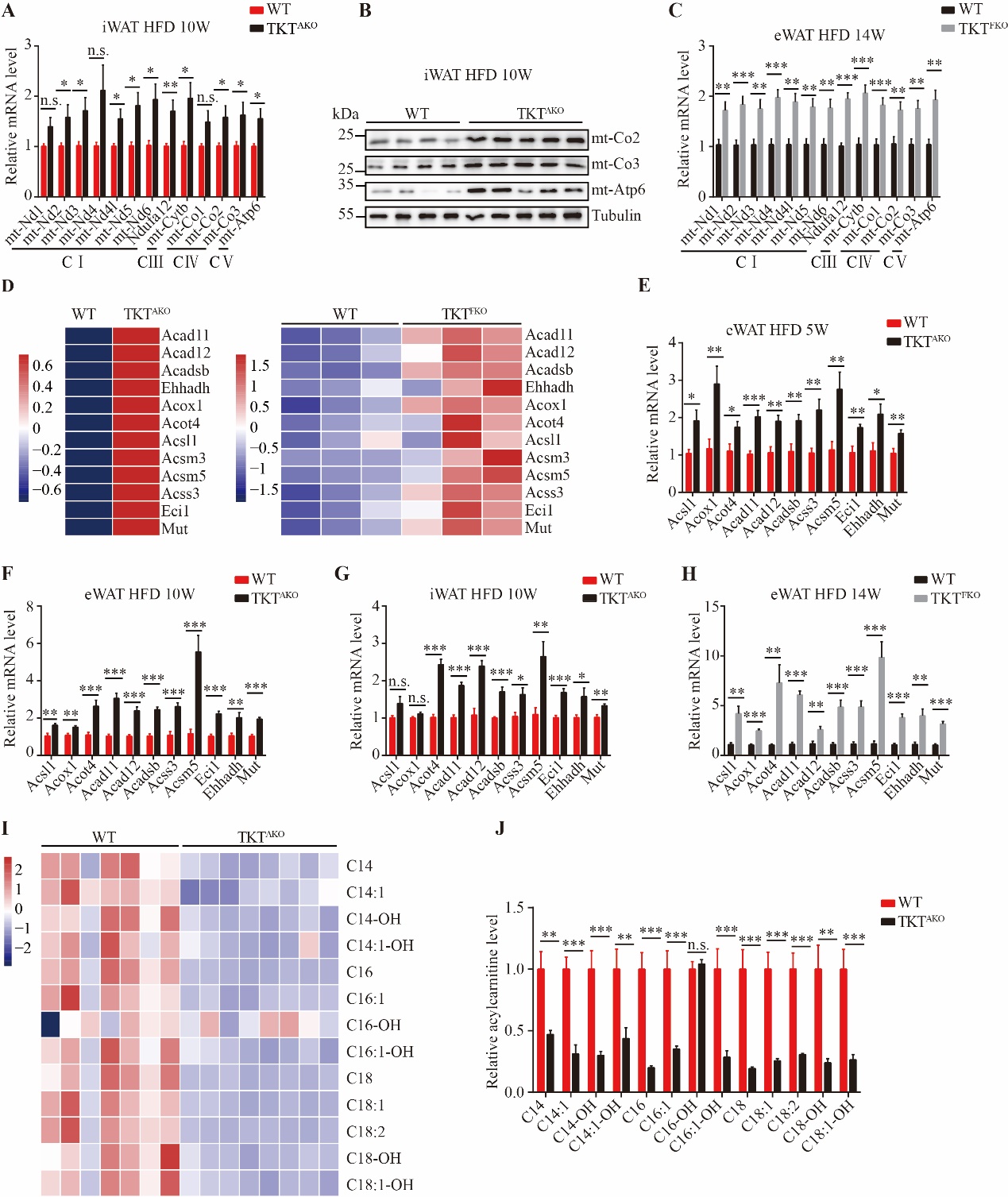
**Supplementary Fig. 3** Loss of TKT in adipose tissues increases energy expenditure. 14-week HFD represents mice fed with HFD from 6-week old for 14 weeks. (*A, B*) Oxygen consumption (VO2) monitored over a 24 h period (*A*) and shown as averaged values (*B*) of WT and TKTFKO mice fed with 14-week HFD (n=8). (*C, D*) Carbon dioxide production (VCO2) monitored over a 24 h period (*C*) and shown as averaged values (*D*) of WT and TKTFKO mice on 14-week HFD (n=8). (*E*) Locomotor activity measured over a 24 h period shown as averaged XTOT counts (left) and YTOT counts (right) of WT and TKTFKO mice on 14-week HFD (n=8). (*F*) Food intake of WT and TKTFKO mice on 14-week HFD (n=5). (*G*) RER of WT and TKTAKO mice on 10-week HFD (n=6). (*H*) RER of WT and TKTFKO mice on 14-week HFD (n=8). Data are represented as mean ± SD. Significance is determined by Student’s *t* test analysis, \**P* < 0.05, \*\**P*<0.01, \*\*\**P*<0.001.



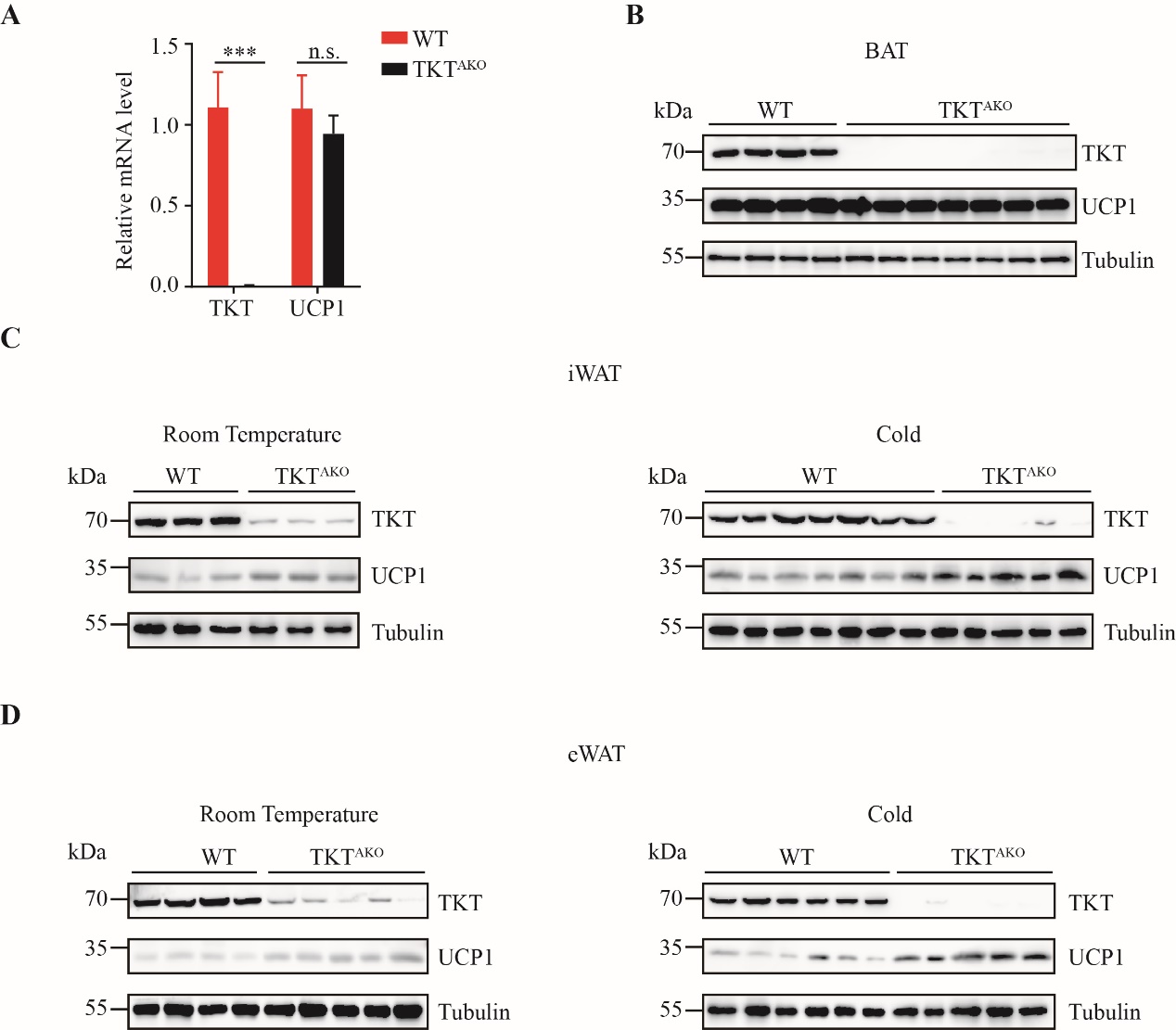
**Supplementary Fig. 4** Adipocyte TKT ablation promotes lipolysis. 3-, 10- or 14-week HFD represents mice fed with HFD from 6-week old for 3, 10 or 14 weeks, respectively. (*A*) Western blotting analysis of protein levels of lipolytic genes in eWATs from WT mice fed with NCD and HFD. (*B*) qPCR analysis of mRNA levels of lipolytic genes in eWATs from WT and TKTAKO mice fed with 3-week HFD (n=7). (*C*) Western blotting analysis of protein levels of lipolytic genes in eWATs from WT and TKTAKO mice fed with 3-week HFD. (*D*) Body weight of WT and TKTAKO mice fed with 3-week HFD (n=7). (*E*) qPCR analysis of mRNA levels of lipolytic genes in iWATs from WT and TKTAKO mice on 10-week HFD (n=7). (*F*) Western blotting analysis of protein levels of lipolytic genes in iWATs from WT and TKTAKO mice on 10-week HFD. (*G*) qPCR analysis of mRNA levels of lipolytic genes in eWATs from WT and TKTFKO mice on 14-week HFD (n=7). (*H*) Western blotting analysis of protein levels of lipolytic genes in eWATs from WT and TKTFKO mice fed with 14-week HFD. (*I*) Western blotting analysis of protein levels in eWATs from WT and TKTAKO fed with NCD and HFD. (*J*) *Ex vivo* lipolysis assay for eWAT explants from WT and TKTFKO mice fed with 14-week HFD in the absence or presence of 10 μM ISO. FFA and glycerol release were measured (n=6 for WT mice, n=7 for TKTFKO mice). Data are represented as mean ± SD. Significance is determined by Student’s *t* test analysis, \**P* < 0.05, \*\**P*<0.01, \*\*\**P*<0.001.



**Supplementary Fig. 5** Adipocyte-specific TKT deletion causes metabolic rewriting. 5-week or 14-week HFD represents mice fed with HFD from 6-week old for 5 weeks or 14 weeks, respectively. (*A*) Schematic diagram of the glycolysis pathway and PPP. The increase and decrease of metabolites are represented by arrows going up and down, respectively. (*B*) Relative levels of adenine, guanine and uric acid in eWATs from WT and TKTAKO mice fed with 5-week HFD (n=5). (*C*) Relative GSH levels in eWATs from WT and TKTAKO mice fed with 5-week HFD (n=5). (*D*) Representative images of DHE staining of eWATs from WT and TKTAKO mice fed with HFD. (*E*) Relative levels of metabolites in TCA cycle in eWATs from WT and TKTAKO mice fed with 5-week HFD (n=5). (*F*) qPCR analysis of mRNA levels of glycolytic genes in eWATs from WT and TKTFKO mice on 14-week HFD (n=5). (*G*) Readcount of Hk1, Hk2 and Hk3 in eWATs of WT mice from RNA-seq data. (*H*) Relative levels of Leu, Ile and Val in eWATs from WT and TKTAKO mice fed with 5-week HFD (n=5). (*I*) qPCR analysis of enzyme genes for branched chain amino acid (BCAA) catabolism including branched chain amino acid transaminase 2 (BCAT2), branched chain keto acid dehydrogenase E1 subunit alpha (BCKDHa) and beta (BCKDHb), and dihydrolipoamide branched chain transacylase E2 (Dbt) in eWATs from WT and TKTAKO mice on 10-week HFD (n=8). (*J*) Western blotting analysis of BCAT2, BCKDHa, BCKDHb and Dbt in eWATs from WT and TKTAKO mice on 10-week HFD. Data are represented as mean ± SEM. Significance is determined by Student’s *t* test analysis or one-way ANOVA analysis. \**P* < 0.05, \*\**P*<0.01, \*\*\**P*<0.001.



**Supplementary Fig. 6** TKT-deficient adipocytes exhibit enhanced mitochondrial function and FAO. 5-, 10- or 14-week HFD represents mice fed with HFD from 6-week old for 5, 10 or 14 weeks, respectively. (*A*) qPCR analysis of mRNA levels of ETC genes in iWATs from WT and TKTAKO mice on 10-week HFD (n=7). (*B*) Western blotting analysis of ETC genes in iWATs from WT and TKTAKO mice on 10-week HFD. (*C*) qPCR analysis of mRNA levels of ETC genes in eWATs from WT and TKTFKO mice fed with 14-week HFD (n=8). (*D*) Heat maps of fatty acid oxidation (FAO) genes in eWATs from WT and TKTAKO mice on 10-week (left) or WT and TKTFKO mice on 14-week HFD (right). (*E*) qPCR analysis of mRNA levels of FAO genes in eWATs from WT and TKTAKO mice fed with 5-week HFD (n=7). (*F*) qPCR analysis of mRNA levels of FAO genes in eWATs from WT and TKTAKO mice fed with 10-week HFD (n=8). (*G*) qPCR analysis of mRNA levels of FAO genes in iWATs from WT and TKTAKO mice fed with 10-week HFD (n=7). (*H*) qPCR analysis of mRNA levels of FAO genes in eWATs from WT and TKTFKO mice fed with 14-week HFD (n=8). (*I*) Heat map of C14-C18 long-chain acylcarnitine species in eWATs from WT and TKTAKO mice fed with 10-week HFD (n=7 for WT mice, n=8 for TKTAKO mice). (*J*) Relative levels of C14-C18 long-chain acylcarnitine species in eWATs from WT and TKTAKO mice fed with 10-week HFD (n=7 for WT mice, n=8 for TKTAKO mice). Data are represented as mean ± SEM. Significance is determined by Student’s *t* test analysis, \**P* < 0.05, \*\**P*<0.01, \*\*\**P*<0.001.



**Supplementary Fig. 7** AdipocyteTKT deficiency does not affect BAT UCP1-dependent thermogenesis but promotes WAT browning. 10-week HFD represents mice fed with HFD from 6-week old for 10 weeks. (*A*) qPCR analysis of mRNA levels of TKT and UCP1 in BATs from WT and TKTAKO mice fed with 10-week HFD (n=8). (*B*) Western blotting analysis of protein levels of TKT and UCP1 in BATs from WT and TKTAKO mice fed with 10-week HFD. (*C*) Western blotting analysis of protein levels of TKT and UCP1 in iWATs from WT and TKTAKO mice fed with 10-week HFD at room temperature and upon cold exposure. (*D*) Western blotting analysis of protein levels of TKT and UCP1 in eWATs from WT and TKTAKO mice fed with 10-week HFD at room temperature and upon cold exposure. Data are represented as mean ± SEM. Significance is determined by Student’s *t* test analysis, \**P* < 0.05, \*\**P*<0.01, \*\*\**P*<0.001.

Supplementary Table S1

|  |  |  |
| --- | --- | --- |
| REAGENT | COMPANY | CAT# |
| Kits |  |  |
| Triglyceride Quantification Colorimetric/Fluorometric Kit | BioVision | K622 |
| Ultra Sensitive Mouse Insulin ELISA Kit | Crystal Chem | 90080 |
| Mouse Adiponectin/Acrp30 Quantikine ELISA Kit | R&D Systems | MRP300 |
| Free Glycerol Reagent | Sigma | F6428 |
| Free Fatty Acid Quantification Colorimetric/Fluorometric Kit | BioVision | K612 |
| Mouse/Rat Leptin Quantikine ELISA Kit | R&D Systems | MOB00 |
| Antibodies |  |  |
| HRP-conjugated α-tubulin | Proteintech | HRP-66031 |
| Anti-TKT | Cell Signaling Technology | 8616 |
| Anti-ATGL | Cell Signaling Technology | 2138 |
| Anti-HSL | Cell Signaling Technology | 4107 |
| Anti-MGL | Abcam | ab24701 |
| Anti-mt-Co1 | Signalway Antibody | 45586 |
| Anti-mt-Co2 | Proteintech | 55070-1-AP |
| Anti-mt-Co3 | Proteintech | 55082-1-AP |
| Anti-mt-Atp6 | Proteintech | 55313-1-AP |
| Anti-Akt | Cell Signaling Technology | 4691 |
| Anti-p-Akt (s473) | Cell Signaling Technology | 4060 |
| Dbt | ThermoFisher | PA5-29727 |
| BCKDHa | Santa Cruz | SC271538 |
| BCKDHb | Santa Cruz | SC374630 |
| BCAT2 | Proteintech | 16417-1-AP |

Supplementary Table S2

|  |  |  |
| --- | --- | --- |
| Primers for qPCR | | |
| Gene name | Forward primer | Reverse primer |
| 18s rRNA | TTGACTCAACACGGGAAACC | AGACAAATCGCTCCACCAAC |
| ATGL | TTCACCATCCGCTTGTTGGAG | AGATGGTCACCCAATTTCCTC |
| HSL | ACGCTACACAAAGGCTGCTT | TCGTTGCGTTTGTAGTGCTC |
| MGL | CGGACTTCCAAGTTTTTGTCAGA | GCAGCCACTAGGATGGAGATG |
| mt-Nd1 | GGCCCTAACATTGTTGGTCC | TGGGTGTGGTATTGGTAGGG |
| mt-Nd2 | TCCTGTTAGTGGTGGAAGGC | CCTTACAACCCATCCCTCACT |
| mt-Nd3 | CTAGTTGCATTCTGACTCCCC | TGCTCATGGTAGTGGAAGTAGA |
| mt-Nd4 | CTAATAATCGCACATGGCCTC | CGTAGTTGGAGTTTGCTAGG |
| mt-Nd4l | TCTTCAACCTCACCATAGCCT | AGATGGTGATGGGGATTGGT |
| mt-Nd5 | CATCCTTCTCAACTTTACTGGG | TTTATGGGTGTAATGCGGT |
| mt-Nd6 | GGGATGTTGGTTGTGTTTGGA | CTACCCCAATCCCTCCTTCC |
| Ndufa12 | TCCTGAGTGGCACCGC | CTCTGGAGTGGCAGACACATT |
| mt-Co1 | ACACAACTTTCTTTGATCCCG | AGAATCAGAACAGATGCTGG |
| mt-Co2 | ATAATCCCAACAAACGACCT | CTCGGTTATCAACTTCTAGCA |
| mt-Co3 | GGTATAATTCTATTCATCGTCTCGG | AGAACGCTCAGAAGAATCCT |
| mt-Cytb | CCATTCTACGCTCAATCCCCA | AGGCTTCGTTGCTTTGAGGTA |
| mt-Atp6 | CCTTCAATCCTATTCCCATCC | GTTGGAAAGAATGGAGACGG |
| Hk1 | GGCCTAGACCACCTGAATGT | CTCTTAGGCGTTCGTAGGGT |
| Hk3 | GTGTGAAGTCGGGCTGATTG | CAGAGTCGAAGGTGGTCAGT |
| Aldoc | AGATGCCCTCCCATGTTTGA | ATAAGGAGCACGAGTCAGGG |
| Pgk1 | TCGTGATGAGGGTGGACTTC | GGCCCACACAATCCTTCAAG |
| Pgam1 | ATCAGCAAGGATCGCAGGTA | CAGATGCTTAACGATGCCCC |
| PKM2 | GTCTGGAGAAACAGCCAAGG | CGGAGTTCCTCGAATAGCTG |
| Acox1 | ACGCCACTTCCTTGCTCTTC | AGATTGGTAGAAATTGCTGCAAA |
| Acsl1 | TGGGGTGGAAATCATCAGCC | CACAGCATTACACACTGTACAACGG |
| Acad11 | GAACATGTGCAGGGTCGGAT | GAATGTAGCCATGCCAGGGT |
| Acad12 | GCGCTGAGTCTTCTGGTTTG | AGATGGAGGCTGCAGAAAGTT |
| Acadsb | TTATGCATCTGAGGTCGCTGG | GGTGTTCAGCTGGATGTTGG |
| Acss3 | CATTGGTGGCAAACAGAGACT | CCAGGTGGTAATGGCAACTT |
| Ehhadh | GGCGCAGGATACCTTGAGAA | TGATTACAGTTGGACTGATGGCA |
| Eci1 | TCTGCCATCAATGGAGCCTCT | GCCCGATGGTGTTCACATAC |
| Mut | CCCAAACACTGACCGTTCTCAT | GCTTCAGGTAATGGGGCGATA |
| Acot4 | GGAGGCCTGTTGGAATACCG | TGGGCCTTTTACCTTTGGATGT |
| Acsm5 | GGCTTCTCCAGAGCACAACT | CCATCCACCTCCTTCCACTG |
| BCAT2 | TTCATTCGTCAGAGCCTGGATA | ACTACTCCAGGCAAGATGACGC |
| Bckdha | AACCAGCCCTTCCTCATTGA | GGCCTTCTCCTGTTCCTCAT |
| Bckdhb | TTCCATGGCCCAAGAAAAGC | AAGATGTGAGGAAACGGGGT |
| Dbt | CAAAGACCGAACAGAGCCAG | ACGGAGGCATTGAGGATAGG |
| Resistin | TTCCTTGTCCCTGAACTGCT | TCTTCACGAATGTCCCACGA |
| Visfatin | CGAGAAGTACAGAGGCACCA | CCACGCCATCTCCTTGAATG |
| RBP4 | GAGCAACTGGGAAGTGTGTG | AGGAGTACTGCAGAGCGAAG |
| Apelin | GAGAAACTGTCCCCTTCCCA | AAGCACTCACCTCCCTACAC |
| Adiponectin | GCACTGGCAAGTTCTACTGCAA | GTAGGTGAAGAGAACGGCCTTGT |
| Leptin | TCATGCCAGCACTCAAAAAC | AGCACCACAAAACCTGATCC |
| UCP1 | GTGAACCCGACAACTTCCGAA | TGCCAGGCAAGCTGAAACTC |

|  |  |  |
| --- | --- | --- |
| Primers for mitochondrial DNA quantification | | |
| Gene name | Forward primer | Reverse primer |
| β-Actin | GGCTGTATTCCCCTCCATCG | CCAGTTGGTAACAATGCCATGT |
| mt-Co1 | TTGGTCCCCTCCTCCAGC | CCAGTGCTAGCCGCAGGCA |