Figure S1

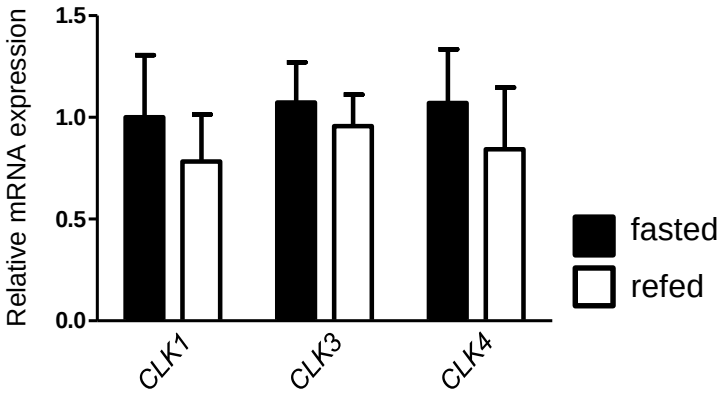

Figure S1: Other CLK family kinases are not regulated on the mRNA level upon fasting and refeeding. Related to Figure 1. Relative mRNA expression of CLK1, CLK3, CLK4 in BAT after fasting (20h) and refeeding (20h fasting, 18h refeeding).

Figure S2

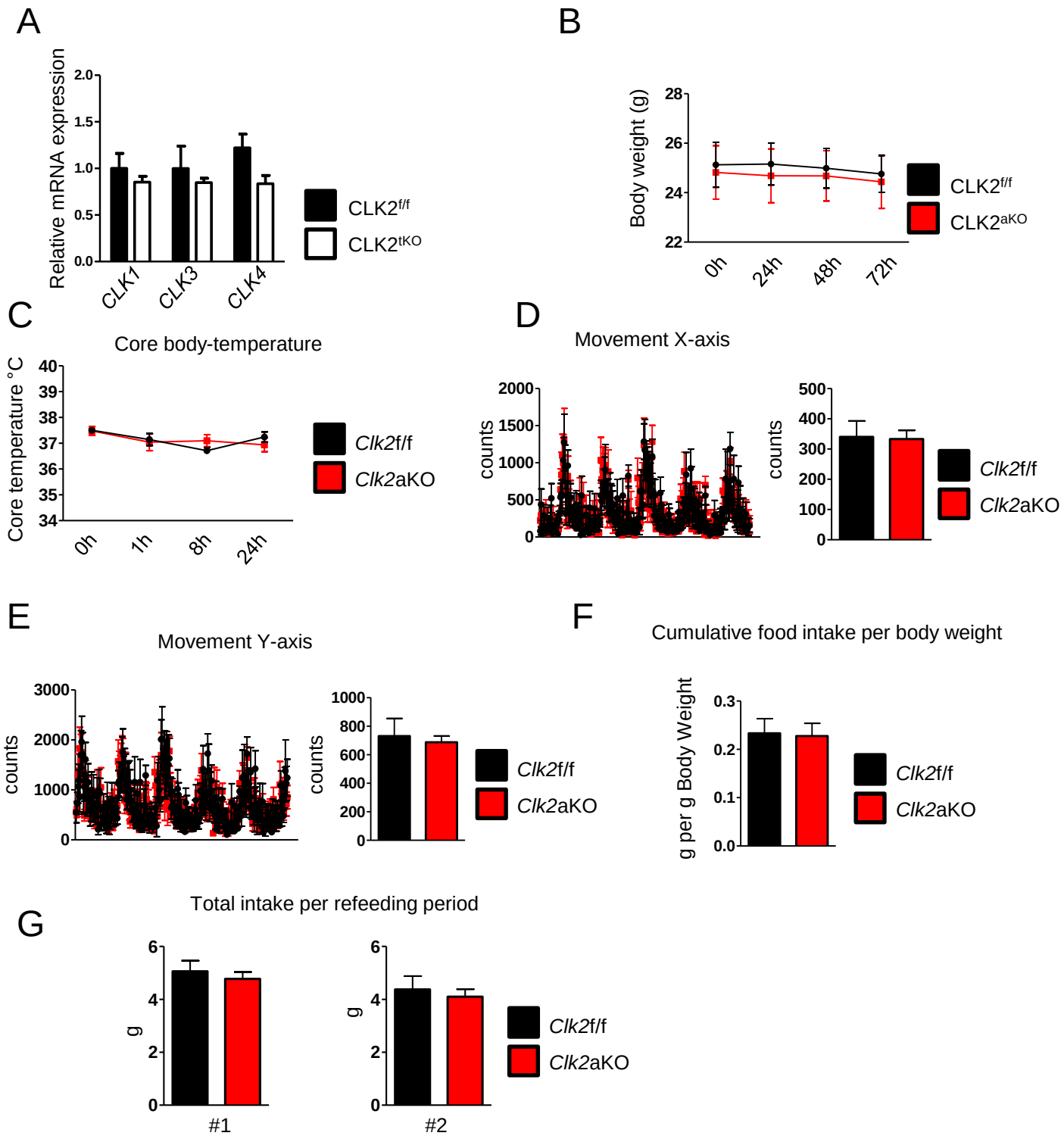

Figure S2: **CLK2 deletion in adipose tissue does not affect gene expression of other CLK family kinases in brown adipocytes, and Clk2 does not alter cold response, movement or food intake in mice. Related to Figure 2.** A. Relative mRNA levels of CLK family kinases in primary brown adipocytes. B. Body weight after acute cold exposure (4°C). C. Core body temperature measured by rectal probe after acute cold exposure. D. Horizontal movement on X-axis of CLAMS chamber. Bar graph represents average counts per hour. E. Horizontal movement on Y-axis of CLAMS chamber. Bar graph representing average counts per hour. F. Food intake presented as indicated. G. Food intake presented as indicated.

Data shown as mean ± SEM. Students t-test (2 data sets) or one-way-ANOVA (multiple data sets) were performed and $p<0.05$ was considered to be significant and indicated with *.

Figure S3

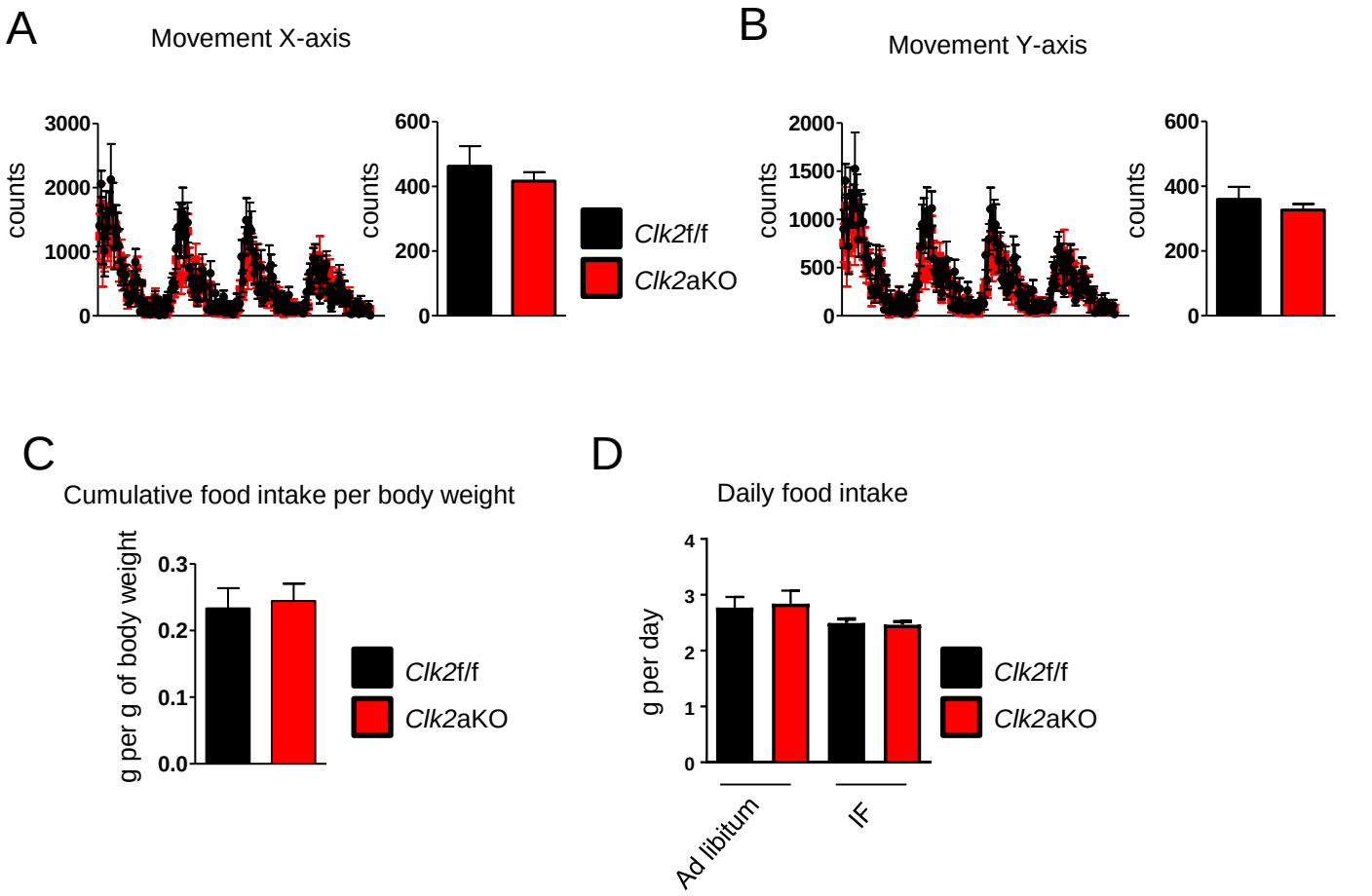

Figure S3: **CLK2 deficiency does not affect movement or food intake during intermittent fasting. Related to Figure 3.** A) Horizontal movement on X-axis of CLAMS chamber. Bar graph represents average counts per hour. B) Horizontal movement on Y-axis of CLAMS chamber. Bar graph represents average counts per hour. C) Food intake per body weight. Left panel shows data from CLAMS experiment, right panels shows data from long-term feeding experiments. Data shown as mean ± SEM. Students t-test (2 data sets) or one-way-ANOVA (multiple data sets) were performed and $p<0.05$ was considered to be significant and indicated with *.

Figure S4

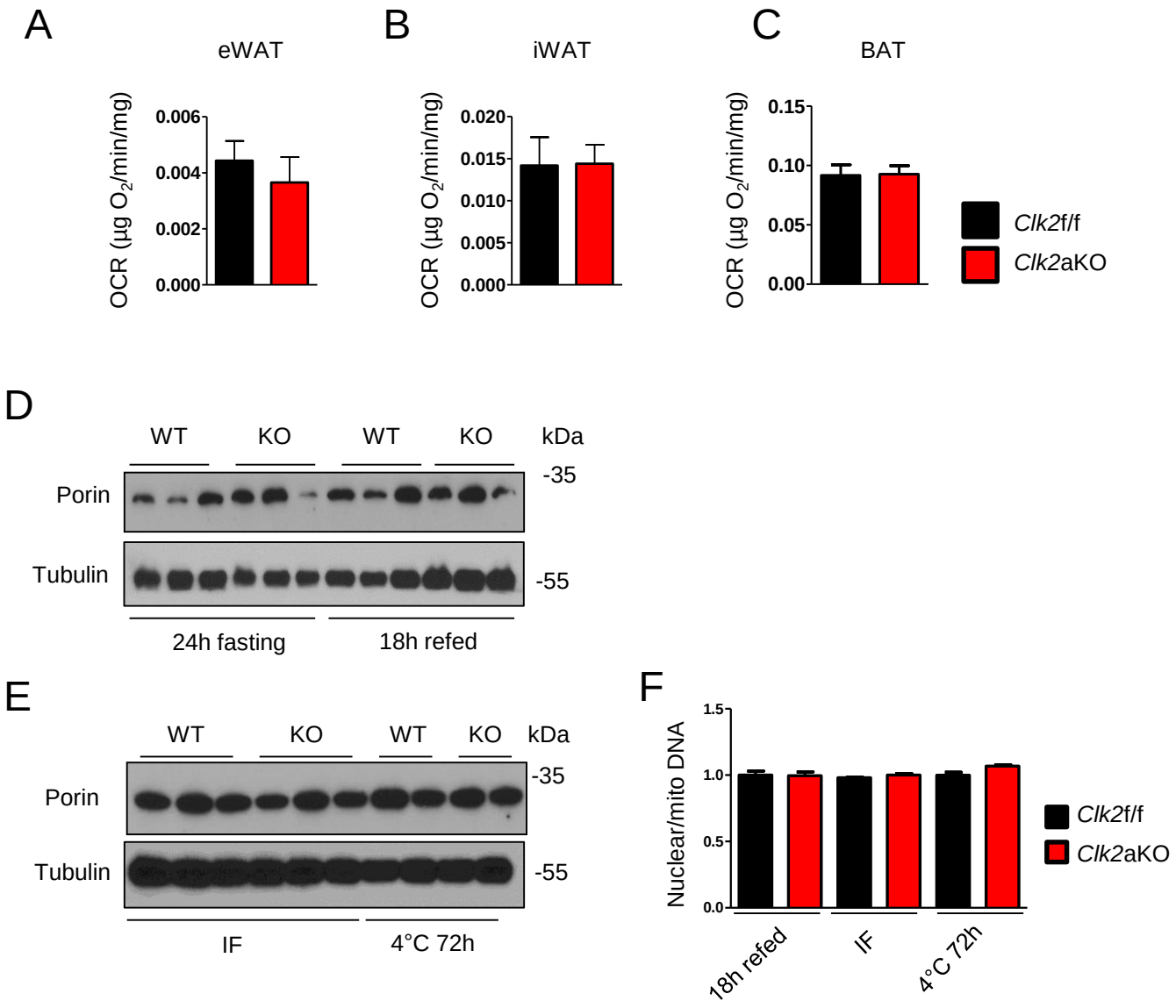

Figure S4: Oxygen consumption is not changed during fasting in CLK2-deficient BAT or white adipose depots during refeeding. Related to Figure 4. A) eWAT OCR as measured with a Clark electrode after refeeding. B) iWAT OCR as measured with a Clark electrode after refeeding. C) BAT OCR as measured with a Clark electrode after fasting. D) Western blot analysis of BAT from lean *Clk2*f/f and *Clk2*aKO mice after 18h refeeding. E) Western blot analysis of BAT from *Clk2*f/f and *Clk2*aKO mice subjected to IF for 12 weeks and control mice housed at 4°C for 72 hours. F) qRT-PCR analysis of nuclear and mitochondrial DNA from BAT with treatments as indicated (n=3). Data shown as mean ± SEM. Students t-test (2 data sets) or one-way-ANOVA (multiple data sets) were performed and $p<0.05$ was considered to be significant and indicated with *.

Figure S5

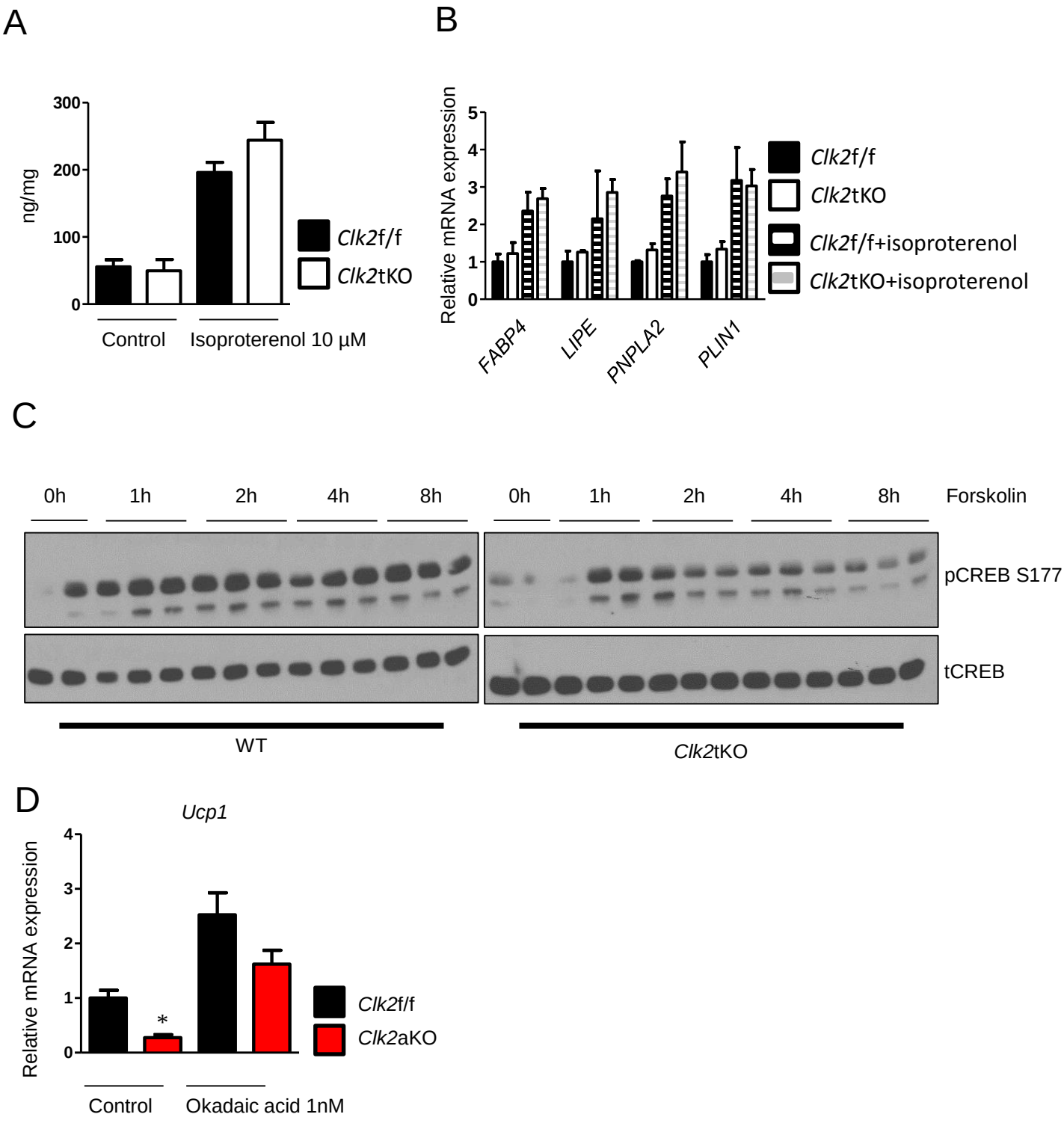

Figure S5: CLK2 does not affect lipolysis in white primary adipocytes, but decreases CREB dephosphorylation in primary BAT cells. Related to Figure 6. A) Free glycerol measured in cell culture media of control primary iWAT cells and cells treated with isoproterenol for 6h. B) mRNA expression in control primary iWAT cells and cells treated with isoproterenol for 6h. C) Western blots from primary adipocytes treated with forskolin at indicated timepoints. D) Relative mRNA levels of UCP1 in primary BAT cells untreated or treated with okadaic acid for 2h at indicated concentration. Data shown as mean ± SEM. Students t-test (2 data sets) or one-way-ANOVA (multiple data sets) were performed and $p<0.05$ was considered to be significant and indicated with *.

Table S1

Gene Name	Forward Primer	Reverse Primer
18s (nuclear DNA)	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
36b4	GCAGACAACGTGGGCTCCAAGCAGAT	GGTCCTCCTTGGTGAACACGAAGCCC
Adiponectin	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
Ap2	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Clk2	CGAAGAAGAAGTCGCTCC	TCCGCCGCCGCCTTGTCC
Cox1 (mito DNA)	AGTGCTAGCCGCAGGCATTACTAT	CTGGGTGCCCAAAGAATCAGAACA
Cox5a	GGGTCACACGAGACAGATGA	GGAACCAGATCATAGCCAACA
Cox5b	TGTATGATTCGTGGTGGAAGCCCT	TTCATGACCTGTCCGCTTGAGTGT
Cox7a	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA
Cox8b	CCGCTTAGTGAACACTCCTTC	TCTACAAACTCTGACAGGGCTTT
Cytc	GGAGGCAAGCATAAGACTGG	TCCATCAGGGTATCCTCTCC
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Elovl6	TCGAACTGGTGCTTACATGC	TGCATAAGCCCAGAATTTGC
Gpat	CAACACCATCCCCGACATCC	GTGACCTTCGATTATGCGATCA
Lipe	CCGCTGACTTCCTGCAAGAG	CTGGGTCTATGGCGAATCGG
Nor1	CGCCGAAACCGATGTCA	TGTACGCACAACTTCCTTAACCA
Pgc1a	AATACCGCAAAGAGCACGAG	ACCAACGTAAATCACACGGC
Plin1	GATCGCCTCTGAACTGAAGG	CTTCTCGATGCTTCCCAGAG
Plin2	GACCTTGTGTCCTCCGCTTAT	CAACCGCAATTTGTGGCTC
Pnpla2	GGTGACCATCTGCCTTCCAG	TGCAGAAGAGACCCAGCAGT
Pparγ	TGTCGGTTTCAGAAGTGCCTTG	TTCAGCTGGTCGATATCACTGGAG
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Ucp1	GGCATTCAGAGGCAAATCAGCT	CAATGAACACTGCCACACCTC

Table S1: List of primers used in experiments. Related to Experimental Procedures.